

Where has all the stuffing gone?

British universities seem ill-equipped to fight back against their most obdurate critic, the government whose instrument of policy on higher education they are supposed to be.

BRITISH universities have had a rotten time in the past few years. Is it possible that their painful experiences have made them incapable of resisting the continuing pressure from their critics, chiefly the British government? That is the charitable explanation of the mood of last week's conference, organized by the universities' trade association, the Committee of Vice-Chancellors and Principals, which was ostensibly intended as a forum for hammering out a reply to the government's green paper on higher education, published earlier this year (see *Nature* 315, 265; 1985). That the committee should have it in mind to reply to the government's tactless document by producing the one that should have been published, cheeky though it may be, is sound. The danger is that the result will not be a defence but a kind of apology, like that of the legendary servant girl who was in trouble for having produced a child of unknown parentage and whose defence was along the lines of "but it's only a small one".

To be fair, the universities seem to have taken account of one of the features of the British educational system that cries out for reform. Several speakers at last week's conference spoke of the need for a broader curriculum both at the secondary schools from which the universities recruit their students and even within universities themselves. It is high time, in the apparently endless brooding about the failure of the British system of higher education, that more serious attention was paid to the value of a liberal education. Indeed, the need that something should be done to make British education less narrow has been urgent at least since the rapid expansion of the early 1960s. For much of that time, British academics have been chief among those seeking to resist change. Only reluctantly, in the past few years, have they been won round to a reform of the pattern of school education that will allow a modest broadening of the education of intending students, no doubt in part because they appreciate that it cannot harm the university system if it becomes more common that students should follow four-year undergraduate courses (as they do already in Scotland). The trend is nevertheless a sign of grace.

Research

On research, the universities seem less sure of themselves. The tenor of the British government's complaint is that too much of what passes for research at British universities is academic in character, unlikely to contribute to national prosperity. This is the spirit in which the budget cuts have been put forward as beneficial incentives to be more practical. Driving academics looking for funds into the arms of industry is bound to make their work more directly relevant to national prosperity, the argument goes. The reply on behalf of the universities last week was curiously muted. Universities have a snobbish disdain for industry (as the green paper complains)? "That cannot be true now; just look at the difficulty we have in persuading graduates to stay on for research!" Universities have been slow to assist with the transfer of technology to industry (another complaint)? "Ah well, things are changing; some universities are making substantial sums of money out of industrial liaisons." The weakness in such replies is that they concede the principles of the complaints the government has been making. It would be permissible, and in the end rather safer, to insist that only individual

universities can usefully shape their own destiny.

What more robust defence can there be? What British universities should recognize is that there is no reason why the system of higher education to which they collectively belong should be capable both of contributing to the development of technology and of preserving the old traditions of scholarship and research, but that the flaw in the British government's complaints over the past six years is that it is unreasonable to expect that each single institution will contribute in the same way. Constitutionally, the vice-chancellors' committee cannot easily abandon the polite convention that all universities are equal, which is especially crippling when it is acknowledged that the essence of an effective reply to the British government's complaints must be a plea that greater diversity is now, as for decades, the most urgent need. The truth is that British higher education has been made too uniform as a consequence of government policies and the polite conventions of the academic life. Only within such a framework will it be feasible for some universities and polytechnics to make their way in the world by concentrating on the needs of industry and for others to do what they already do well, scholarship and academic research. But will not greater diversity flow from the plan of the University Grants Committee to begin allocating a part of its subvention for universities on the basis of a judgement of the quality of the research which is at present carried out? The first steps in this direction will have been taken six months or so from now. The most obvious danger is that the result will be to strengthen the universities that are already strong, and publicly marking out the others as second-rate, undeserving of more than basic support and thus incapable of self-improvement.

Budget

The impending row about this discriminatory allocation of money is only one of the several novel crises now facing British universities. Another is the continuing decline of the budget, estimated to be shrinking still at between one and a half and two per cent a year when allowance is made for increased costs and the difficulty of settling salary increases within the government's national allowance. The University Grants Committee has said that it will not be long before it is forced to recommend that one or more institutions should be closed, but that it will not shoulder the invidious task of saying where the axe should fall. That crisis could come sooner rather than later, perhaps as early as next May. Then, to round out the sense of gathering gloom, the function of the grants committee itself is being reviewed. The excuse is a minor reference in a report on the efficiency of universities published earlier this year, but it seems unlikely that the committee appointed for the purpose will constrain itself within a framework of accountancy. The ideal is that the review should settle on principles for supporting all kinds of higher education, polytechnics as well as universities, and should then work out a way in which the grants committee can function more as a monitor of success or failure than as a source of funds. But who will say what is success? On last week's showing, the vice-chancellors are not up to the job. Nor, by the evidence of this year's green paper, is the British government. Might that not be the lasting function of a grants committee with a broader membership? □



Crossing the water

France and Britain now seem bent on building a physical link. Will it really happen?

THE bids announced last week by the consortia wishing to build a physical link between Britain and France have been widely predicted and are, as far as they go, staid and safe. The Channel Tunnel Company, which already owns a number of holes in the ground and beneath the seabed on the English side of the water, is understandably bent on building a tunnel, a rather better one than that abandoned in the early 1970s. Others are variously concerned to build a bridge the whole way across this turbulent 21 miles of water, or to span just over half of it with bridge-like structures that then disappear beneath the sea in a stretch of tunnel that will allow shipping to pass without hindrance. The tunnel, which would accommodate only trains, is the cheapest project (at roughly £2,500 million). The bridge-tunnel plan is roughly twice as expensive; building a bridge the whole way across (with an accompanying railway tunnel with which to placate the nationalized railway systems on each side of the Channel) works out at half as much again. The only certainty about what will happen next is that the governments of France and Britain will decide on the scheme to be allowed to go ahead, but that the construction costs will have to be met by private interests, banks and the like.

So has the time arrived when the future of the Channel tunnel link will be laid to rest? Most probably not. For the best part of two centuries, schemes to build a Channel link have been canvassed enthusiastically and, then, as suddenly abandoned. The fear that Napoleon might be able to march an army through a tunnel brought the first wave of enthusiasm to a halt. Now, not even fears of cultural invasion can upset the case for building a link of some kind across the Channel, which is crudely economic. All the bidders now in the market for the attention of their governments base their arguments on the assumption that the cross-Channel business will be worth £500 million a year or so by the time that a tunnel or a bridge is built. If the estimates of cost now put forward are anything like correct, each of the schemes should be economically viable at some point in the 1990s. But how likely are the planners to be proved correct? And what will happen if their estimates are seriously in error, and if the project chosen in the next few months runs into trouble, geological or financial, in the succeeding years?

Whatever the two governments may say, they cannot wash their hands of some kind of responsibility for whichever project they choose. From the start, they will be intimately involved, as governments, in the renegotiation of navigation rules between France and Britain. And on each side of the Channel, the project will need planning permission on a scale that only governments can provide. Then, in reality, there is no chance that either government could turn its back if a project turned sour after a few years. At the very least, it might be necessary to dismantle a few surplus structures in the centre of a busy international waterway. But the political pressures that would apply to government that decided it could abandon a physical link halfway through construction would be irresistible.

That is one reason why there is the strongest case for the two governments to face the responsibilities they will not be able to escape now, and not to wait until the project is under way, with money committed. It is right and proper that they should expect the bulk of the funds required for the project to be raised from private sources, but it would help to give the project solid foundations if the two governments were to become minority shareholders in their own right. Then, if the worst came to the worst, they would be in a position to decide whether to step in to mount a rescue for a faltering project or to continue to stand aside.

Meanwhile, it remains to be seen which project will be chosen. Given their mutual interests in the prosperity of railways, both governments have a vested interest in the rail tunnel, but this should be suppressed. The high economic value of the

traffic between Britain and France, and the pace at which it is growing, shows that the trade transcends the narrow interests of the railway industries. To be able to drive across as well as go by train should be the objective, whence the case for the hybrid bridge and tunnel at the very least. The misfortune in all this, however, is that so little has been done to implement what is obviously the best of all ways of crossing the Channel, the scheme for putting the clock back to the late Pleistocene, when lots of people crossed from France to Britain, and building a dam across this narrow strip of water, nowhere more than 120 feet deep. □

No tin-pan alley

The International Tin Council, an international cartel, is in trouble. The surprise is the delay.

Two weeks ago, the London Metal Exchange was thrown into chaos by the announcement that the gentleman called the buffer stock manager of the International Tin Council had run out of money. Trading in tin was promptly suspended, and has not yet been resumed. Meanwhile, commodity brokers in the City of London, used as they are to skating on thin ice, are worried sick by the prospect that some of them may not be able to pay the debts they have incurred over the past several months in buying and selling tin. Even the British government, in its avuncular role as the custodian of the last resort of the reputation of the City for honest dealing, is alarmed that the reputations of people and of institutions may be so damaged that people elsewhere will not use London as a place in which to buy and sell metals and other commodities. (London has a virtual monopoly on metal-trading.) The several causes of the tin crisis have by now been well catalogued. Improbably, they range from the illicit activities of poachers based in Singapore who have been dredging tin from the deep waters of Malaysia to the cupidity of the tin-producing countries, which have done everything they can to keep up the retail price of tin, including the curious practice of buying back through the Tin Council roughly a third of all the metal their producers have been able to sell. Collectively, the tin producers and their customers have been united through the International Tin Agreements, by whose terms the International Tin Council exists, in an attempt to ensure that water runs uphill. In the past few days, the natural laws have reasserted themselves.

Nobody should be a whit surprised. All along, the objective of the Tin Council has been to stabilize the price of tin. The buffer stock manager has instructions to buy the metal when the price falls below a certain value (fixed periodically) and to sell when the price exceeds another value. In between, however, he is neutral, sitting on the stocks he has accumulated. Although the United States has remained stolidly outside the tin agreements, other consumers have over the years benefited almost as much as the tin producers. Prices have indeed been stable, much more than might be expected in a market in which the balance between supply and demand can fluctuate enormously. The trouble is that, as in the petroleum market, stability means different things to producers and consumers. In tin, the chief producers, among whom Malaysia is the most important, have naturally taken the view that stability is not worthwhile unless it also implies a high price, just over £8,000 a tonne when trading was halted ten days ago. But the industrial demand for tin has shrunk as the many importers of this expensive metal have learned to use others instead. In retrospect, it is clear that the buffer stock manager should have blown the whistle earlier, and that he should not have waited until he ran out of credit with the banks to whom the tin market now owes the best part of £600 million. Rarely can the buffer stock have grown as consistently as in the past few months. But the diagnosis is easier after the event. And now, when nothing is sure except that a great many people and banks will be left holding a large quantity of tin they do not want, the lesson to be learned from this discreditable episode is that the laws of supply and demand are not safely defied. □

AIDS

Politics of premature French claim of cure

THE French physician who claimed last week a dramatic improvement in patients with acquired immune deficiency syndrome (AIDS) when they were treated with the immunosuppressant cyclosporin A now says he was pressed into a premature press conference by an over-enthusiastic minister.

"It wasn't our decision — it was the ministry of health's", said Dr Philippe Even of the Laennec Hospital in Paris. Dr Even agrees that the announcement of results after a week's trial with six patients, without controls, was "premature", but claims it was the pressure and the enthusiasm of health minister Mme Georgina Dufoix that led to the press conference. Dufoix appeared on television to claim this was "a great French discovery" that "bears the label of France".

Even is now following the line of his critics, saying that what is needed now is a full-scale trial. Ironically, it was his request to the ministry for help in setting up a trial that led to the widely-condemned public announcement.

Nevertheless, Even says, the treatment of his key patient, a 38-year-old man acutely ill with "end-stage" AIDS, has been "really dramatic". This person had been the most seriously ill of six patients treated, by last Friday, for nine days with the drug. The patient has shown a 100-fold increase in the population of T-4 (T-helper) cells, the white blood cells specifically infected by the AIDS virus. Now the man has a T-4 cell count of 400 per cubic millimetre, against 4 previously. Having been comatose and bed-ridden, he is now walking and feeding himself.

Dr Even also claims that "something very curious" happened during treatment indicating that cyclosporin A has a genuine effect. The patient, who had been taking cyclosporin orally got diarrhoea on the sixth day of treatment which interfered with absorption. His T-cell count immediately dropped, so that Even and his colleagues Jean-Marie Andrieu and Alain Venet decided to administer the drug intravenously. "Immediately his T-cell count rose again", says Even.

The five other patients, all less acutely affected, had shown at least a doubling in T-cell count since treatment to levels of more than 300 per cubic millimetre (against a healthy level of some 700–1,200).

Even's explanation is that cyclosporin A halts the activation of T-4 cells but does not inhibit the production of new T-4 cells by the bone marrow and the thymus gland. The result, says Even, is that mature T-4 cells can be held in check while

the immune system regenerates itself. Even says that the activation of T-4 cells is "pivotal" in the replication of AIDS virus and the development of the disease; he speculates that an occasional course of cyclosporin A might keep the disease in check or even, with luck, eliminate it.

Outside France, AIDS clinicians say that the premature release of results was not only improper but cruel; "some of our patients will clutch at anything". There is also a fear that cyclosporin A could in the long run actually exacerbate the disease. According to one leading immunologist, the drug alone mimics the immunosuppressive effects of AIDS itself. There are also cases of AIDS-antibody-positive renal transplant patients who were put on immunosuppressants subsequently developing more acute AIDS than patients who had not been immunosuppressed, it is pointed out in London.

Moreover, the rise in T-4 cell count seen in Paris appears too rapid to be explained by regeneration of lymphocytes by the

bone marrow, a process that might be expected to take 3–4 weeks, rather than just nine days. Further, the cerebral infections seen in AIDS patients, perhaps mimicking the infections of sheep by visna virus, may be resistant to cyclosporin A, London immunologists point out. The target cells for the HTLV-III/LAV virus of AIDS in the brain have not yet been identified, and may not be controlled by cyclosporin A even if it crosses the blood/brain barrier.

But Dr Even says that not one in five of the population of new T-cells in the patient's blood is an immature T-6 cell from the thymus, indicating that cyclosporin A has stimulated the production of new lymphocytes, at least from the thymus gland.

The next step, says Even, will be the setting up of a multi-centre trial of cyclosporin A in acute patients in France and, later, of an international trial. The pharmaceuticals company Sandoz, which makes and markets cyclosporin A, is convening an international conference on 8 November at its Basle headquarters in Switzerland, to prepare just such an international trial. But the only North American delegates attending will be Canadian, Even says. So far there are no delegates from the United States, and therefore no apparent US interest in joining in a trial. "That is their decision", Even says.

Robert Walgate

SDI

Star wars critics criticized

Washington

THE US Congress's Office of Technology Assessment (OTA), no stranger to controversy over President Reagan's Strategic Defense Initiative (SDI), has run into yet another dispute on the subject. Dr Robert Jastrow, an independent authority on missile defence, and Dr Frederick Seitz, chairman of the Defense Science Board, last week protested that the majority of an OTA advisory panel that supervised a recent OTA report* on SDI (see *Nature* 26 September, p. 276) were "strongly opposed" to SDI, and that OTA staff were biased against the project.

One of Jastrow and Seitz's chief complaints is that the OTA advisory panel, of which Seitz was a member, did not vote to endorse the report, and that there was no outside review. OTA's report concluded that SDI will increase national security only if the project achieves great technical success and if the Soviet Union cooperates in a negotiated arms reduction. Peter Sharfman, OTA's international security programme manager, replies to the criticism by pointing out that OTA's advisory panels never vote on staff reports and that their function is to provide independent review.

The Jastrow/Seitz attack on OTA was made at a briefing held by the Heritage Foundation, a conservative think-tank with strong links with the Reagan adminis-

tration. Seitz was recruited to the advisory panel after General Daniel Graham, a strong advocate of SDI, had resigned, also alleging bias. Sharfman rejects categorically the charge that a majority of the panel were "strongly opposed" to SDI, and denies the suggestion that OTA staff were stretched beyond their limits.

Apart from the procedural issues, Seitz and Jastrow say that OTA failed to take account of numerous technical advances in missile defence in the past year. In their view, progress has been so encouraging that key demonstrations of SDI technology might be feasible 10 years sooner than was expected, with deployment possible during the 1990s. Among the advances named are the free electron laser, electromagnetic railguns and a new type of rocket engine, SCRAMJET.

OTA replies that it was familiar with the technological advances but avoided giving performance details because they are classified; all the technologies mentioned by Seitz and Jastrow, excluding SCRAMJET, are described in the OTA report. And in reply to a criticism that OTA overestimated the effectiveness of Soviet countermeasures, OTA says it regrets that specific technical objections were not raised by the advisory panel before the report's publication.

Tim Beardsley

*Ballistic Missile Defense Technologies. Office of Technology Assessment, 1985.

Export controls

Scepticism over liberality promise

Washington

THE US Department of Commerce (DoC), long the bugbear of US high technology exporters because of its allegedly inefficient processing of export licence applications, is to make substantial changes in its licensing regulations. The changes are required by recent legislation and by agreements reached with the United States' partners in COCOM, the Coordinating Committee on Multilateral export Controls*.

Most recently, the department has been advertising to industry the virtues of its new "foreign availability office", intended to ensure that the United States does not restrict on national security grounds exports of high technology items that are freely available to the Eastern bloc from other sources. But, logical though this sounds, industry is sceptical of whether the office will have the desired effect.

Foreign availability was supposed to be taken into account in determining whether to allow exports even before the Export Administration Amendments Act became law on 12 July, but requests for licences on these grounds usually fell on deaf ears. The new office is intended to change that. William Archey, assistant secretary for trade administration, has described the new foreign availability rules as "the dark horse" of export regulations.

Under the new act, after receiving an export licence application that rests on a claim of foreign availability, DoC will be allowed a grace period of 18 months during which time the US government can seek to "negotiate away" the foreign source of the item in question. The exporter has to establish that the item is available in comparable quantity and quality from a foreign source, but the burden of proof will now lie with DoC if it seeks to reject the applications. Even then, DoC may not be able politically to allow the export of some items demonstrably of foreign availability. Yet, even if the cynics are right, the office may still benefit industry in other ways.

Bill Maxwell, of the Computer and Business Equipment Manufacturers' Association, believes that the new office will probably succeed in preventing items being added unnecessarily to DoC's "commodity control list" of proscribed items, even if it does not manage to remove any already there. And that could be significant, especially with increasing sophistication of electronic equipment manufactured in some other "Pacific rim" countries.

The United States has consistently made the running in efforts to restrict the flow of Western high technology equipment to the East. Last year, DoC turned down licence applications for \$300 million worth of exports for which orders had been received, on the basis of pre-licence

checks and intelligence reports. But the United States unilaterally controls exports of some items that are not controlled by its major trading partners, which has frequently led to quarrels with allies.

One especially sensitive issue has been whether contracts legally entered into can subsequently be terminated for foreign policy reasons by invoking export controls. Despite lobbying by allied governments, nothing in the new act alters the presumption of extraterritoriality. The new act will nevertheless make it slightly harder for the President to terminate contracts for foreign policy reasons. He has to testify that the controls are necessary, and that a failure to act will lead to a "breach of the peace". (What exactly constitutes a breach of the peace is left undefined. The resulting loophole is "big enough to drive a truck through", according to one independent specialist.)

Among COCOM's more significant achievements was the agreement last year to decontrol exports to the Eastern bloc of many less-capable computers, on the grounds either that they were unlikely to be of military use or that control was impractical. COCOM merely has to be notified when exports of this sort are planned, and DoC has recently introduced regulations to allow such exports from the United States to COCOM member countries without a licence.

But if the decontrolled items will reduce DoC's workload, other changes required by the new act will increase it. DoC has been given tight new timetables for processing licence applications: 15 days for exports to COCOM countries and 60 for most others for which COCOM review is necessary (down from 90). The timetables will benefit US exporters — if they are met.

Walter Olson, deputy assistant secretary for trade administration, says the United States is "pleased" by progress in COCOM, although he notes there are still areas where the United States and other members do not yet see eye to eye.

Disputed items include certain kinds of software, particularly that used in designing and fabricating semiconductors. But Olson hopes that further progress will be forthcoming, especially now that COCOM has changed its cycle of operations so that one quarter of the entire (secret) proscribed list will be reviewed each year, rather than there being a complete review every three or four years. The result, he says, will be that DoC will be able to keep effective controls on those items that need to be safeguarded, while ensuring that some of the complaints that have been heard in the past about US manufacturers hobbled by harsh export controls are not repeated.

Tim Beardsley

* COCOM member countries are the United States, the United Kingdom, France, Belgium, Italy, the Netherlands, Luxembourg, Norway, Denmark, Canada, West Germany, Portugal, Greece, Turkey and Japan.

Texas keeps on bidding for collider

Washington

COMPETITION among states to play host to the proposed Superconducting Supercollider (SSC) has led to questions in Congress about design assumptions even before a complete conceptual design has been drawn up. When the central design group announced in September its choice of magnet design for the accelerator, most people thought the question had been settled. The group chose a high-field cos θ design over a competing superferic design (see *Nature* 26 September, p.277). But Representative Joe Barton (Republican, Texas) has now carried out his own study, and concludes that the design team made a mistake.

Both designs employ superconducting magnets, but where the high-field cos θ design relies on the arrangement of the current-carrying wires to produce a uniform magnetic field, the superferic design relies partly on steel pole pieces to shape the field and return flux efficiently. The superferic magnet design is cheaper per foot, but because it produces a lower-strength field more magnets are needed. One important factor in a choice between the two designs is the cost of tunnelling, which depends on the terrain.

The superferic design would lead to a

larger SSC with a circumference of 100 miles, as opposed to a modest 60 miles with high-field magnets. Texas would be ideal for SSC, especially if the larger version were to be built, for the state has sites where tunnelling costs would be low. Last week, at a hearing in the House of Representatives on prospects for high-energy physics, Mr Barton told Maury Tigner, head of the design group, that "it could be shown that" the superferic design would lead to a cheaper SSC. Tigner hotly disagreed, offering to debate with Barton, point by point, on the magnet selection panel's analysis. Barton said he might submit some written questions.

With Congress in a deficit-cutting mood, winning a commitment to build SSC will be difficult. The US high-energy physics community last week brought in big guns from Europe to help make their case, in the form of Herwig Schopper, director of CERN in Switzerland, and Carlo Rubbia, at present at Harvard University. Present estimates of the cost of SSC, a 40 TeV proton-proton collider, range from \$3,000 to \$6,000 million. The design group should complete its conceptual design study in April, when more accurate cost estimates should be possible.

Tim Beardsley

Plant engineering

Gene transfer makes a start

Savannah, Georgia

ONE reflection of the rapid growth of plant molecular biology is that it is now possible to attract some 1,800 participants to one of those unwieldy international symposia whose concurrent sessions occupy the ballrooms of the larger hotels in out-of-season resorts. The International Society for Plant Molecular Biology at any rate regards its congress held here last week as a milestone in the field.

Although the use of recombinant DNA to transfer genes between plants may eventually change the face of the Earth, to judge from last week's scientific presentations, its principal impact in the immediate future is likely to be on the fortunes of the agrichemical industries that support much of the basic research. Only two weeks ago, a team from Calgene reported (*Nature* 317, 741; 1985) the introduction into a tobacco plant of a bacterial gene conferring resistance to the Monsanto herbicide glyphosate; similar results were reported here by Rob Fraley (from Monsanto's own laboratories working in collaboration with Nam-Hai Chua and colleagues at Rockefeller University), but with a plant gene and in tomato as well as tobacco and petunia.

Glyphosate is an indiscriminate herbicide that destroys crop plants and weeds alike, and whose use is therefore confined to ground clearance. Glyphosate tolerance into crop plants would thus greatly extend the application of the chemical.

But this is not the sole, or probably even the principal, reason for the impressive advances in the manipulation of herbicide resistance. Analogous experiments were reported by Lawrence Bogorod (Harvard) with a gene for the Ciba-Geigy herbicide atrazine, which is now out of patent and so no longer of compelling commercial interest. The attraction is that the genetic and biochemical basis of herbicide tolerance is both relatively simple and extremely well understood. Most broadly desirable genetic assets, such as the ability to resist pathogens or fix nitrogen are complex and relatively ill-understood. In pathogen resistance, pathogens seem capable of producing virulent mutants about as fast as molecular biologists can engineer plants to resist them. Herbicides are an easier target.

Among other sobering considerations offered in a presentation by M.S. Swaminathan, director of the Institute of Rice Research in Manila, is that the basic genetics of Third-World staples such as rice are in such a primitive state that they have simply not been accessible to molecular manipulation. A further difficulty is that rice, like other cereals, is a monocotyledon. So far, the recipes for regenerating complete plants from manipulated cells will work only for dicots, whence the con-

centration on tobacco, petunias or, at best, tomatoes.

Nevertheless, the Rockefeller Foundation is supporting a campaign on the rice genome which, with the use of the molecular mapping techniques that have been so successfully exploited in the analysis of human genetic disease, should be reasonably well charted within a year or so. Progress on regenerating genetically engineered plants is less predictable (tissue culture is still largely a black art) but two speakers at Savannah reported a significant step forward. Genes artificially introduced have not in the past functioned stably in cultured cells from monocotyledons, but this has now been achieved in maize (Michael Fromm, Stanford) and Italian rye grass (Lawrence Potrykus, Friedrich Miescher Institute). The next step is to persuade the cultured cells to

regenerate into a complete plant, which many believe will be a matter chiefly of luck and patience.

Meanwhile, new techniques for gene transfer based on electroporation (which allows DNA to enter cells exposed to strong electric fields) are helping molecular biologists to identify and isolate the genetic control elements that determine the responses of plants to the critical features of their environment. The DNA elements that enable genes to be activated by heat and light have already been identified; Jim Peacock reported that his group at the Commonwealth Scientific and Industrial Research Organization in Canberra now has a gene that controls the induction of others in anaerobic conditions. In principle, these genetic control elements could be manipulated to increase the resistance of crops to natural disasters such as drought. How it will be in practice, and when, it is too soon to guess. Even resistance to herbicides is not yet ready for the field. **Miranda Robertson**

Space insurance

Market confident of survival

Washington

US GOVERNMENT intervention to provide emergency insurance cover for commercial space ventures would be disastrous. Witnesses from the National Aeronautics and Space Administration (NASA), insurance companies and satellite manufacturers all agreed on this point when telling Congress last week how to prevent a space insurance crisis; more than \$600 million has been lost by insurers in the past 21 months, threatening the much vaunted commercialization of space.

James Barrett of Intec (International Technology Underwriters) is confident that market forces will prevail and allow the insurance industry to continue to "facilitate, but not subsidize" commercial space projects. He suggests spreading the load of space insurance risks throughout many smaller companies, which could support an "insurance pool" dedicated to space shuttle launches and cargoes. He also feels that the manufacturers should take more of the risk for the functioning of their products, which would doubtless improve reliability. Barrett is opposed to the sort of competition that is occurring at the moment between Arianespace and NASA; because Arianespace provides guaranteed launch insurance at below-market rates, NASA is planning to offer cut-price relaunches to customers whose satellites have failed within a given time-span. Such deals will deprive the insurance industry of premium revenue and inhibit the spread of risk, according to Barrett.

Jack O'Brien, NASA's general counsel, agrees that the market will stabilize without government intervention. NASA

already helps its smaller customers by waiving liability for small self-contained payloads so that the only loss the experimenters face is the face value of the project. NASA also disallows claims against each other by participants in a particular flight, and includes itself in this ban. It is carrying out a further evaluation of its risk allocation policies, but wants to find a solution in conjunction with the insurance industry that does not open the door for government regulations.

Predictably, the manufacturers themselves do not want to be liable for any loss risk. Charles Schmidt of RCA Astro-Electronics says that the fiercely competitive business leaves no room for such guarantees. Smaller companies would lose business if a "no insurance, no financing" policy were introduced. One compromise would be for the industry itself to form a last-resort pool.

The message to the government from the construction industry and the insurers alike is clear — the market will look after itself. The satellite industry was so successful in the first ten years of its existence that insurers have grossly underestimated the cost of risks, leading directly to the present crisis. Underwriters are convinced that the pendulum will swing back and that a stable compromise can be reached if the government will give them time. In a country where space is taken so seriously that the normally conscientious chairman, Bill Nielson (Democrat, Florida), of the committee taking last week's evidence was absent because he is in training for the next shuttle flight, the commercial space industry had better make sure it has reason for its confidence that a solution can be found. **Maxine Clarke**

Japanese conservation

War on war on migrating birds

Tokyo

JAPAN may not have much of reputation abroad when it comes to protection of wild life. But inside the country, conservation groups are growing stronger. In the coming weeks, volunteers from the Wild Bird Society of Japan will be taking to the hills in force to try to prevent the mass netting of migratory birds.

During middle to late November, migrating birds pour into northern Japan to escape the Siberian winter. In olden days, many were netted out of sheer necessity;



the farmers of the northern provinces were poor and the birds provided one of the few sources of protein in winter. Now that Japan is an advanced technological power, poverty has disappeared and there are not many people left in the bird netting business. But advanced technology has also made it possible for a few people to catch an enormous number of birds.

Nowadays, Japan makes the world's finest mist nets — light, strong and so fine that they are invisible to the bird's eye — and plenty of inexpensive audio systems that can be used to broadcast recorded bird calls. The result is that flocks of thousands of birds may be drawn into a large net and captured at one time. Those most frequently taken are *tsugumi* (dusky thrush, *Turdus naumanni*), *kashiradaka* (rustic bunting, *Emberiza rustica*) and *atori* (brambling, *Fringilla montifringilla*). Last year alone it is estimated that more than four million birds were killed and mostly sold to restaurants. And as a thrush can fetch as much as 1,000 yen (nearly US \$5) it is a profitable business. Recently it has attracted the interest of criminal gangs.

The setting of mist nets has actually been illegal since 1947, but as there is no restriction on their sale and fines for illegal taking of birds are small, the law has had little effect.

Increased concern for wild birds is now beginning to make inroads into the poachers' takings. The membership of the Wild Bird Society of Japan has increased

seven or eight times since the end of the 1970s to its present 16,000. This year, for the first time, volunteers from the society will patrol the hills all through the key November weeks, searching for illegal mist nets. If any are found, the evidence is guarded and the police called.

The society would like to take things a step further and ban manufacture of mist nets altogether. That would please environmental groups in Italy and Spain which are fighting a mass capture of migrating birds in their own countries. Mist nets are imported from Japan. The Environment Agency, which has been pressing local governments to crack down on the poachers, is also backing a ban on mist nets. But the agency lacks regulatory powers and has yet to move the ministry responsible, the Ministry for International Trade and Industry (MITI). So far, MITI has tended to take the manufacturers' side, for a variety of rather spurious reasons, including the possible effect of a mist net ban on the hair net industry.

Alun Anderson

New Soviet guidelines

THE new draft programme of the Communist Party of the Soviet Union, the first since 1961, includes not only a strong commitment to scientific and technical progress, but also a code of conduct for scientists.

The party, it is stated, "supports the bold quest, the rivalry of ideas and approaches in science, and fruitful debates and discussions". Contraindications for science therefore include: scholastic deliberations, the passive recording of facts without drawing bold generalizations, jumping on bandwagons, and severing oneself from reality. In view of the complex and interconnected nature of current problems, a deepening of the links between the natural, technical and social sciences is required. Forms of organization of science should be developed that will facilitate interdisciplinary study of topical problems and the necessary mobility of scientific cadres. The structure of scientific institutions and of research and development procedures must be made more flexible.

An "essential condition for the progress of science", it is noted, is the constant influx of fresh forces, including forces from the production sector, and the skilful utilization of scientists' creative potential.

Finally, and tacitly admitting that ideology is not enough to get and keep Soviet research working at full capacity, the programme specifically urges the provision of financial incentives for scientists "depending on their real contribution to the study of theoretical and applied problems".

Vera Rich

French ceramics

Plans for silicon carbide fibres

HERMES, France's answer to the US space shuttle, will be made of ceramic composite, and will thus avoid the sticky tiles that caused complications in the early days of the US craft. At least, those are the plans of Société Européenne de Propulsion (SEP), the company charged with preparing the curved nose, leading edge of the wings and underbody of Hermes, the parts that will face re-entry temperatures of up to 1,600 degrees centigrade for periods of some 20 minutes.

SEP has chosen for the task one of its most advanced ceramics, consisting of silicon carbide fibres bonded into a sintered silicon carbide matrix: "a kind of ceramic concrete", according to one British ceramicist. A major problem with such materials is to produce curved forms, and Hermes is of course full of aerodynamic curves. But SEP has been working on similar composites, including carbon-carbon matrices, for a decade, and has already produced trial curved silicon carbide/silicon carbide sheets some 50cm-1m across. This is a long way from the many metres required for Hermes sheets, but SEP reckons it has another ten years to develop the technology. If all goes well with the development, the ceramic will be more than mere cladding and will act as a structural part of the craft, although it may need support from more conventional stress-bearing structures underneath.

Meanwhile, according to M. Roger Lesgards, president of SEP, the company's first priority will be to establish French independence in the supply of the silicon carbide fibres, which at present come from Japan. SEP intends investing jointly with the French chemicals giant Rhône Poulenc in a factory to produce the fibres and the composite. SEP did the same with carbon fibres a year ago, in setting up a factory near Lyons to manufacture the fibres in concert with the steel company Alstom Atlantique.

Lesgards describes SEP as having a "really good position" in the world market for high thermal resistance composite ceramics. Japan asked SEP for samples of the silicon carbide/silicon carbide matrix, says Lesgards, and the US Department of Defense is interested in using the material for missile nose-cones. The composite is already in use as brake disks for Formula One racing cars, and is also being tested for use as vanes to direct the thrust of rocket motors.

SEP foresees three possible world markets for the material. The first, requiring production levels of a few tonnes per year, would be a market for space and military uses. The second, raising the market tenfold, would be in aeronautics (for example, in turbines). The third and largest,

involving another tenfold increase, would involve uses in diesel motors and other common equipment, which could raise demand to a few hundred tonnes a year, Lesgards estimates.

The material is costly to produce, but economies of scale could reduce cost tenfold at the highest market levels, Lesgards predicts. With this in mind, SEP recently entered into cooperation with the German diesel engine manufacturers MAN to develop ceramics for diesel motors, with support from French and German government agencies.

SEP also plans to extend its international cooperation to work on ceramic turbines with companies in Norway, Sweden, Spain and Italy, if support is forthcoming from this week's ministerial meeting on Eureka at Hannover. Under Eureka, SEP has put forward a plan for a FF 200 million (£18 million) research programme on ceramic turbines. The company has also proposed the development of a commercial demonstration ceramic diesel engine (FF 100 million) and two varieties of ceramic turbine (FF 100 million each). SEP would be happy if half of the total FF 500 million were supported by governments or banks, Lesgards says. **Robert Walgate**

British nuclear research

Harwell goes half-private

In the UK Atomic Energy Authority (AEA), 1 April 1986 is known as Vesting Day. This is Civil-Service-speak for the day on which AEA ceases to be a division of a government department and becomes a "trading fund", equivalent in status to that of a limited company in which the British Treasury is the only shareholder. Among other things, the authority will thus be able to lend and borrow money on the open market. The Treasury will set financial targets, and there will be high hopes on the government side that the authority will be able to generate a surplus.

AEA's largest establishment, the research laboratory at Harwell in Oxfordshire, is also the best placed to profit from the new regime. A year ago, an encouraging 33 per cent of its research was supported by non-governmental contracts, but now the figure is nearly 60 per cent.

To continue at its current level of activity, Harwell needs funds totalling £100 million a year. From next April, there will no longer be a subvention from the De-

partment of Energy (DoE) to cover research carried out on behalf of government departments and other parts of AEA. Instead, separate contracts will be negotiated to cover each research programme — including fast reactor research, fusion research and work on thermal reactor and general safety research. It does not follow that the individual contracts will together compensate for the lost lump-sum contribution, the two being calculated in different ways, which is another stimulus for Harwell to look further afield for contracts.

Recent investment in the facilities at Harwell should help in the push for more outside contracts. A new radiochemical handling laboratory has been built, based on experience gained at Los Alamos in the United States and at Karlsruhe and Jülich in West Germany. The five high-integrity concrete cells can contain any combination of alpha, beta and gamma radiation. Each cell is ventilated separately so that a chemist measuring, say, a caesium isotope in one cell need not fear that someone else's experiment is generating just that activity at the other end of the containment area. Experiments are set up in large stainless steel boxes, or "cassettes", before being hoisted into the containment cell on cranes.

The new laboratory was opened last week by Sir Frederick Dainton, on his last day as chairman of the National Radiological Protection Board. The date was chosen to mark an anniversary of sorts — 40 years ago, on 29 October 1945, Prime Minister Clement Attlee announced in the House of Commons that Harwell, a disused airfield near Didcot, was to become Britain's first nuclear research laboratory.

Main customers for the new facilities will be British Nuclear Fuels Limited, the Central Electricity Generating Board, other AEA divisions and Amersham International, the radiochemicals company spun off from AEA several years ago. Although the company is now branching out into the increasingly popular field of non-radioactive assay kits, Amersham's isotope business is still large, and will be an important part of Harwell's plans, managed through Amersham's own offices on the Harwell site.

While Harwell can contemplate a future of at least the same level of activity as now — provided that in the long term it is able to adapt to the changing needs of industry — some other AEA divisions have fared less well in recent government reviews. Spending on fusion research at the Culham laboratory, for instance, is to be reduced by £1 million, £2 million and £3 million in three successive financial years. However, support for the Joint European Torus (JET), also sited at Culham, is not affected. **Charles Wenz**

New Soviet space agency is formed

THE Soviet Union has established a new space agency to make use of space research results in the national economy. The existence of the Main Administration for the Creation and Use of Space Technology for the National Economy and Scientific Research (*Glavkosmos*) was revealed last month in an unremarkable middle-page interview in *Izvestiya* with the new agency's head, A.I. Dunaev. This reticence is all the more remarkable because, when the Soviet press daily castigates President Reagan's strategic defense plans, *Glavkosmos* might have been used as evidence of the more peaceful Soviet approach to space.

Reticence, however, seems to be the keynote of *Glavkosmos*. The interview does not reveal when it was established, only that it "has been set up and has started working". Dunaev, however, refers to all its activities in the future tense, suggesting that it is a very recent innovation. Dunaev's own identity, too, remains obscure. Nothing is said of his career or academic qualifications, his background or even his given name and patronymic.

Even the purpose of *Glavkosmos* itself remains obscure. Dunaev notes the benefits of space research to the Soviet economy; long-distance communications (including the simultaneous printing of Moscow newspapers in Siberia and the Far East), meteorology, land-resources survey, navigation and the international "Kosmos-Sarsat" air-sea rescue system as well as the production of new superpure and biologically active materials aboard manned spacecraft. But his list reads like a

summary of the Soviet presentation at the United Nations conference on the peaceful uses of space (UNISPACE-82) three years ago.

Within the Soviet Union, the main purpose of *Glavkosmos* seems to be administrative. A number of ministries and government departments, Dunaev said, are concerned as are scientific organizations.

A special coordinating body is now needed, and *Glavkosmos* will have charge of the planning and space projects for the collection and dissemination of space information for practical purposes, and will also be responsible for Soviet participation in international cooperation programmes, both the Comecon "Interkosmos" project, and broader programmes such as the current *Veha* (Venus/Halley) probes in which nine countries are cooperating.

Whether *Glavkosmos* will be responsible for Soviet manned programmes is unclear. Dunaev's reference to future long-term stations is part of a general outline of space plans, not of the specific functions of his agency. Also unclear is the relationship of *Glavkosmos* with the Space Research Institute of the Soviet Academy of Sciences, which so far has acted as coordinator for the transmission of space data to the production sector.

Glavkosmos will not, it appears, be responsible for research planning, and its creation seems merely to interpose one more body between research and production. But it is precisely here that endemic delays in the transmission of results occur.

Vera Rich

Soviet computer education

Teething troubles galore

SOVIET plans to introduce computer studies in the senior forms of every school throughout the Soviet Union may be delayed by up to six years, according to the Uzbek historian, U. Mannanov, a member of the joint commission on information science and computer technology. Launched by a resolution of the Council of Ministers of the USSR and the Communist Party central committee in March, the plan was one of the first major decisions of the Gorbachev era, and was widely acclaimed as the next significant step in implementing the "scientific and technological revolution". Computer studies were already operating on a pilot scale in Soviet schools, but the essential feature of the new plan was its universality — every school was to have microcomputers, and every 15- and 16-year-old would learn how to use them.

Fifteen pairs of blue jeans and a personal computer.....?



From the beginning, there seemed to be some doubt as to whether the hardware would be ready in time. At the end of March, the director of the Institute of Teaching Content and Methods of the USSR Academy of Pedagogic Sciences, Professor Vadim Monakhov, admitted that the choice of computers to install would be determined by availability, not suitability. In the event, many schools failed to get their computers and, when the new term began in September, were informed that they would have to teach "theoretical" computer studies until the hardware arrived. At the same time, leading articles in *Pravda* hailed the introduction of universal computer studies as a major advance in the building of a truly science-orientated society, implying that the delay in installing the computers was a fairly short-term one. Mannanov's revelation of the likely length of delay was made only in *Pravda Vostoka* (Eastern Truth), a provincial newspaper not normally available in Moscow.

Another problem is a shortage of teachers able to teach computer studies. During the vacation, summer schools were organized for the physics and mathematics teachers who would be taking the new courses. According to Mannanov, of those attending the courses

in the Uzbek SSR, 92 per cent had previously never seen a computer.

Although the logistic problems of computer education are obviously worse in the Soviet Union, where editions of textbooks (and now of software packages) have to be envisaged in editions of 4 million, several Comecon countries seem to be experiencing similar difficulties. Poland, with barely a thousand computers in schools, has declared a moratorium in practical computer education for at least two years. Even so, it seems that a large number of pupils are already computer-literate, either from official youth club facilities or through access to "privately acquired" Western computers, of which there are thought to be between 25,000 and 60,000 in the country.

Czechoslovakia, on the other hand, in

spite of an admitted 40 per cent shortfall in deliveries of computers to secondary and vocational schools, has declared computer education one of the two major priorities for the current academic year (the other is peace studies). In Bulgaria, one of the main producers of computers in the Comecon bloc, not all schools even in the capital, Sofia, have their own computers, and some use facilities which properly belong to the higher education sector.

Only in Hungary does computer education seem to be running to schedule. The Hungarians, moreover, seem more aware than many of their Comecon partners of the broader applications of classroom computers, so that during his visit to Britain last month, the Hungarian minister of education and culture, Dr Bela Koepecsi, particularly requested a visit to a secondary modern school where computers are used not only in the mathematics and science classes but also in the humanities.

Vera Rich

Japanese psychiatry

Hospital reforms under way

Tokyo

THE first steps towards reform of Japan's mental hospitals were taken last week with the publication of a new set of guidelines by the Ministry of Health and Welfare. Criticism of the way hospitals are run has been mounting for some years but the ministry showed little reaction until visits from human rights organizations, including the International Committee of Jurists (see *Nature* 316, 571; 1985), stimulated international criticism.

The chief aim of the new guidelines is to give patients in mental hospitals the right to communicate with the outside world. The almost total isolation of mental patients has been seen as a major factor in allowing recent abuses to take place. At the Utsunomiya Hospital, where violence was regularly used against patients and two were alleged to have been beaten to death, patients tried to communicate with the outside world by launching paper aeroplanes. Earlier, an incident at an Osaka hospital where a patient was beaten to death by hospital staff came to light only through the chance discovery of a message from one of the patients. He had written the message on a piece of paper, wrapped it around a stone and thrown it out of the hospital grounds.

Three new provisions are made: public telephones will be installed in closed wards and patients will be free to use them as they wish; patients will be allowed to correspond with anyone they like, including lawyers and human rights groups; and staff will not be present when visitors are received, unless specifically invited. Furthermore, it is recommended that the telephone numbers of human rights organizations be freely displayed.

Critics are far from satisfied: they point

out that the new guidelines are just guidelines and fear they will not be obeyed. They also stress that loopholes exist: communication can be limited if required for medical treatment or protection of patients, while letters may be examined if there is any suspicion that patients might, for example, be trying to send drugs out of the hospital. The only counter to possible misuse of these clauses is that any restrictions placed on patients must be entered on their medical records.

What many groups pressing for reform would like to see are the kinds of changes requested by the International Commission of Jurists, chiefly the setting up of a completely independent review tribunal that would have the right to inspect mental hospitals and to which patients would have right of appeal. At present a patient may be committed to a mental hospital by the superintendent of a mental hospital once the guardian's consent has been obtained and patients have no rights to demand independent medical evaluation. This problem may be tackled in a reform of the Mental Health Act promised by the government for next year.

Whatever legal changes are made, there will remain the difficulties of effecting the changes in attitude that lie at the root of the problem. Although a reform movement exists, and a small number of "half-way houses" and workshops have been set up to help patients back into society, the mentally ill remain outcasts. A quarter of the patients in mental hospitals do not receive even one visit a year. And as many as half the patients could be released from the hospitals if their families were willing to have them back. It is these attitudes that will eventually have to be tackled.

Alun Anderson

Research in Ecuador

Universities are squeezed

Quito, Ecuador

THE Galapagos Islands, long a symbol of ecological diversity and evolutionary theory, also symbolize the relative strength in Ecuador of the biological sciences, blessed both by rich natural resources on their doorstep and by government support.

Dr Tjite de Vries, director of the biology department at Quito's Catholic University, manifests the optimism born of sufficient funds. Each of the three government agencies that support research supports one of his projects (in the highlands, the tropical rain forest and the Galapagos). Moreover, his grant from the Canadian Wildlife Service to study the effect of pesticides on the peregrine falcon exemplifies the flow of foreign aid into biological research in Ecuador. "If a scientist has the initiative", says Dr de Vries, "the money is there", both for equipment and the high logistic costs of working in the rainforest.

The most important governmental agency for university research is El Consejo Nacional de Universidades y Escuelas Politecnicas (CONUEP). Of the 7,000 million sucres (100 sucres=US\$1) distributed in 1985 from the Ministry of Finance to the 17 universities and polytechnics, 500 million sucres were assigned to research. Some research contract work is handled as well by El Consejo Nacional de Ciencia y Tecnologia (CONACYT), but research in the Galapagos is supported by the Charles Darwin Foundation, whose resources come from a mixture of government and foreign sources.

In 1984, CONUEP accepted 97 projects from 13 universities, out of 248 applications. There were as many applications in 1983, but only 25 were supported. Winning government aid depends on doing research in a priority area, as defined by El Consejo de Desarrollo (CONADE) in the National Development Plan 1984-88. Preference is given to projects in health and nutrition, agriculture and technology.

Researchers in Ecuador seem cheerfully to accept government-imposed directions for science. There is no secret about the priorities, which many feel are justified for Ecuador's development. For example, Dr Joan Llauger, head of the chemistry department at the Catholic University, shows no regret at leaving his speciality in metallurgy and concentrating on nutritional questions such as the oxidation of vegetable oil. The government has no history of giving funds to research, explained Mr Juan Maldonado, head of the Charles Darwin Foundation, so researchers have not yet come to consider such aid as their right rather than privilege.

Ninety per cent of government aid flows through the state universities, so the assets of the five private universities are particularly squeezed. Even in Dr de Vries' de-

partment, the highest degree offered is between a US bachelor's and master's. More infrastructure, says Dr de Vries, is necessary to reach international parity, primarily in libraries (the Catholic University cannot afford *Nature*), equipment, field stations and computer facilities. Fields dependent on expensive equipment, such as physics, are much harder hit. The physics department at the Catholic University only prepares teachers; it does no research. Dr Ernesto Cadena of that department says that even though a medical physics graduate programme has been started with foreign aid, there are not enough good physics teachers in high schools, no money for research laboratories, and few jobs for physicists in Ecuador.



Students interested in a higher degree are often encouraged to go abroad and then return to work. Dr de Vries, who is Dutch, came to Ecuador with UNESCO. Two members of his staff got doctorates in the United States, one in Germany, and another received a master's degree in Italy. Some of his students are on fellowship in Britain, the United States and Brazil.

The lack of tradition of research on an international level is the worst problem. Dr Llauger says he invites foreign professors to lecture and presents papers abroad every year, but that few of his colleagues (far fewer than in biology) do likewise. Few universities require publication from the professors.

The plethora of universities in such a small country is itself a doubtful boon. According to Dr Gonzalo Cevallos, who has taught economics at Quito's Central University and now works for the oil firm Intrade, the philosophy of free education has resulted in every town wanting a university. There are no entrance requirements, only a small percentage graduate, and few of these can find jobs.

"Ciencia aplicado" that augments the country's productivity is a clear government priority over basic research. Governmental entities perform much of this work, though, according to many, they are merely applying foreign techniques

rather than pursuing applied research. Funds from the state budget (for 1986, 201,000 million sucres) flow through the various ministries to institutes and government companies, which, says Dr Cevallos, do "research in brackets". INIAP, for example, is an institute under the Ministry of Agriculture that does plant research such as in cross breeding, and CEPE is an oil company controlled by the Ministry of Natural Resources and Energy. The military also has applied research arms: its Geographical Institute, for example, does topographical research, primarily mapping.

The techniques and equipment used by these institutes and companies, if not actually developed in Ecuador, are often the most advanced available. The Geographical Institute uses both US LANDSAT and French satellite facilities; the oil industry uses three-dimensional seismic technology; and the forestry department is importing a tissue culture laboratory to clone trees.

Oil and its byproducts are a primary source of funds for Ecuador's development. Because of its reliance on oil to support government projects and pay off foreign debt, Ecuador will not abide by OPEC's quota of 184,000 barrels/day and is instead nearing 300,000 barrels/day. Even in oil, says Dr Cevallos, it is more economic to use foreign techniques than to try to catch up to international levels of expertise.

Oil also helps to support the growing commitment in Ecuador to learning about the country's environment and how to use it wisely. According to Mr Maldonado, the government has accepted the foundation's advice on resource management, as evinced by an increase in support from an average of 70,000 sucres in 1964-66 to 10-15 million in 1966-85. Sixty wardens have been added to the national park system this year; the peasants are asking for reforestation projects; and a research station in the Amazon basin is studying how to raise alligators, fish and guinea pigs without destroying the forest. Ecuador and Costa Rica, says Dr Roque Sevilla, director of the National Forestry Programme, are the most advanced countries in this part of the world in terms of ecologically sound resource development.

Applied science is then, finally, paramount in Ecuador. That can be explained by the government's "practicality" and the need to spend money elsewhere.

There is another, darker possibility, however. Fear is spreading that the government, backed by banking and manufacturing interests, plans to strangle the universities, which are seen as catalysts of anti-government activity. One commentator says that recent increases in university research funds are nothing in the face of 35 per cent inflation: the government, a democracy only since 1979, "is out to crush the Left with no holds barred".

Elizabeth Collins

NERC studentships

SIR—Keith Runcorn (19 September, p.196) has obviously cogitated carefully over the report of the Natural Environmental Research Council (NERC) committee chaired by me which reviewed methods of allocating research studentships, since his letter appeared nearly a year after the report was published. His views are reminiscent of some of the evidence we received from heads of departments (possibly a slight majority), but which were opposed by a majority of other members of staff and by virtually all the students themselves. Our conclusions were not entirely maverick; we advertised our review as widely as we could and had about 400 comments from academics, employers, students and other research councils about the most equitable and positive ways of allocating studentships. Our recommendations were based on assessing all the information we had, giving appropriate weight to the relevance of the suggestions and criticisms.

It is of little general interest to respond to all the misapprehensions accumulated by Professor Runcorn. Perhaps the drafting of our report was obscure. But five points need making:

(1) The committee consisted of three Earth scientists, two former research council secretaries with backgrounds in the biological sciences (Sir John Gray of the Medical Research Council and Sir William Henderson of the then Agricultural Research Council, both of whom had overseen allocation procedures very different from that used by NERC), and myself, an ecological geneticist. We examined the past and present methods used by all five British research councils, and other possibilities suggested by our respondents. We were unanimous in our recommendations, and our report was endorsed by the NERC Council.

(2) Professor Runcorn opines that "the ratio of geophysical to geological studentships is too small both for scientific advance and for national needs". This is an appropriate and fashionable prejudice for a geophysicist to hold. The underlying problem is that it is extremely difficult to determine the proper allocation of studentships between disciplines. If demand is any guidance, requests in the NERC area for life science studentships are much greater than for Earth science ones. My committee recommended an "in-depth independent study with a view to laying down guidelines for the distribution of available awards between the (subject) committees", and this has been accepted by the NERC Council.

The general and very important problem of forecasting "national need" was analysed by a White Paper on Postgraduate Education (Cmd 6611, 1976) which expressed the view that "it is unlikely that a close match between supply and demand

could ever be achieved and it will be important to resist the temptation to forecast and plan with spurious precision". Sir Peter Swinnerton-Dyer's ABRC Working Party (1983) reviewed past endeavours on the subject and concluded that all these "attempts at manpower planning (for the supply of scientists and technologists) foundered principally on an uncritical faith in the economic contribution of education and on a confused concept of demand. . . since there are many PhDs whose working career has borne little relation to the subject of their thesis however potent the contribution of research training has been to their intellectual and practical development. It remains a truism that the quality of highly trained manpower is as significant as its quantity."

(3) Professor Runcorn repeats the accusation raised originally by the heads of university zoology departments that membership of a NERC committee improves the chance of getting a studentship project approved. If true, this would be worrying. The fact is that some committee members are more successful than expected in some years and less successful in others. There is no consistent picture, but (as Professor Runcorn notes) NERC "Council takes a very serious view (of this) . . . important area which will be carefully monitored in the future". There need be no mystery or suspicion here: if the most active and productive research workers constitute the grants committees, they would be expected to obtain a higher than average proportion of awards. Perhaps the key factor is that the grants committees are not self-perpetuating cabals: for some time NERC has been inviting university departments and individuals to nominate to the committees, and this invitation was repeated in the committee's report. For the record, five of the 18 members of NERC's current geological sciences training awards committee are geophysicists.

(4) Professor Runcorn wants NERC to abandon its "effortful, cumbersome, expensive and unsatisfactory scheme". Ignoring his fourth adjective, our published response to this comment was that "as each research studentship costs in excess of £17,000 and represents three very important years of a student's life, the expenditure of a supervisor's time (in preparing an application) is fully justified".

(5) However, I suspect that the crucial difference between Professor Runcorn and my committee is over the purpose of a research studentship. We firmly and explicitly rejected the idea that a PhD student should be primarily a research assistant involved in an ongoing research project. Professor Runcorn quotes disparagingly "NERC's statement of the primary objective of a research studentship". Our understanding and conclusions were

based on two clear objectives. I am unrepentant about these:

"The primary aim of Council's policy must be to enable the intellectually most able graduates to receive the best possible training. An essential part of this training is the preparation of a PhD thesis, which also provides a means of ensuring that research undertaken is preserved and can be made available to others. Within the context of research training, it is council's aim to influence the quality of training and its direction and content.

"The second main objective of council's policy can be defined as ensuring that there is an adequate supply of manpower trained at postgraduate level to meet the national requirement in the sciences most closely concerned with the natural environment.

"A very important consequence of research training, even though incidental to that training, is the major contribution which research students make to the research output of universities. In itself, provision of training awards is a major contribution to the research community."

I am not clear why Professor Runcorn is so dismayed by our report. As a scientist, he cannot logically complain about an exercise that examines all relevant data and then excludes inadequate conclusions from it (even if he does not like the positive solutions that emerge). If it is any comfort to him, I was not allocated a NERC studentship this year, despite the fact that I put forward two *excellent* proposals to the grants committees. . .

R.J. BERRY

Department of Zoology,
University College London,
London WC1E 6BT, UK

Human life

SIR—The distinction between dependently viable protoplasmic life and independently viable organismal life (respectively at the level scale of the molecule and of the individual), as made by R. C. Hoult (*Nature* 8 August, p.480), is quite correct, although it does not follow that the life quality necessarily attributable to a fetus *in utero* is that of protoplasmic life only.

The fetus has undoubtedly an organismal life, and we can speak of "fetal behavioural states" (*Prenatal Diag.* July 1985, p.269) in spite of its dependence on its mother for energy supply. An infant seems not to differ very much in this respect. It must be recalled that, up to the onset of implantation, the human conceptus is "free living" in the sense that it is not attached to the mother in any way. Nor can I see how a human adult individual during parenteral nutrition could lose his "human life" or organismal quality.

M. ZATTI

Universita degli Studi di Verona,
Centro Ospedaliero Clinizzato,
Istituto di Chimica e Microscopia,
37134 Verona, Italy

The virtues of single atoms

Success in cooling single atoms to millikelvin temperatures, and then of trapping them for seconds (or even hours) on end, will benefit not merely spectroscopists but the rest of physics.

THE obvious benefits of being able to study single ions or even atoms are those that the spectroscopists have been extolling for several years, ever since it seemed probable that finely tuned lasers might be used to rob moving atoms of velocity in a specific fashion. Earlier this year, there was a flurry of excitement with the first demonstration that sodium atoms can be "cooled", or robbed of velocity, by the selective absorption of photons (and thus momentum) of a suitable frequency. (The technique and the consequences were fully discussed by B.W. Petley in a *News and Views* article on 27 June this year, p.716.)

Especially because it is now possible to trap electrically charged particles in suitably configured static electric and magnetic fields, the spectroscopists have had plenty to look for, not least the chance to trap small numbers of ions in excited states so long-lasting that the radiation they emit is neither made fuzzy by the Doppler effect, a consequence of movement, nor broadened substantially by quantum considerations, Heisenberg's uncertainty principle in particular. The notion that suitably prepared trapped atoms may make more convenient standards of frequency (or of time) than the more familiar atomic clocks of caesium is not entirely out of court.

But even before these opportunities have been exploited, the hunt seems well under way for a technique for trapping neutral atoms and molecules in apparently empty space. The first informal report of success in this direction came from Harold Metcalf of the State University of New York at Stony Brook (also in *Nature* on 27 June, p.717) hinting at experiments by his own group and by another at Bell Laboratories in which neutral sodium atoms had been successfully trapped for periods of time measured in seconds. Now it seems that more systematic ways of achieving these objectives are in sight.

The article by R.V.E. Lovelace, C. Mehanian, T.J. Tommila and D.M. Lee, all of them from various parts of the physics enterprise at Cornell University, is not so much the report of a discovery as a challenge to other experimenters. They explain that the goal they have set themselves theoretically is to define the circumstances in which it would be possible to trap atoms of neutral hydrogen and then to explore the question whether such an assemblage could ever be made dense and cool enough so as to undergo the

Bose-Einstein phase transition expected of all bosons, particles (in this case atoms) with integer spin (zero included).

Will the challenge be taken up? A glance at Table 1 on p.31 will show how formidable a task lies ahead. At 0.1 degrees above absolute zero (or at 0.1 K), it would be necessary to compress hydrogen atoms dealt with singly to roughly the density expected from molecular hydrogen gas at normal temperature and pressure. Doing this in a vacuum will require a substantial magnetic bottle. But how can such a device, familiar enough in the manipulation of some plasmas which are candidates for thermonuclear fuel, be made to function for atoms which have no electric charge? The clever trick that Lovelace *et al.* describe entails exploiting the potentially different orientations in a magnetic field of the nuclear and electron spins of single hydrogen atoms. It should be possible to trap those atomic arrangements in which both the nuclear and electron spins are parallel to an external magnetic field by exploiting their tendency to congregate at the places where the field is greatest. The snag is that collisions between atoms would require that the experiment would be feasible only at a temperature of 0.0001 K, thought still to be unattainable. Whence the neat proposal that an oscillating magnetic field should be superimposed on a static field in such a way as to create an oscillating magnetic bottle to pump atoms with different hyperfine structure to different regions.

It remains to be seen whether the scheme proposed by Lovelace *et al.* can be exploited as they suggest, but the fact that the scheme has arisen at this stage is a proof of how far the physics of single atoms has developed in the past year or so. Whatever may be the defects of this scheme, nobody would have thought it sensible to put it forward five years ago. And it is also plain that the interest of schemes for trapping single atoms and molecules, or small numbers of them, extends far beyond spectroscopy. B.W. Petley explained in June how measurements of stable confined atomic systems could be used to test the isotropy of gravitational inertia. T. Erber and S. Putterman, on p.41 of this issue, raise another set of questions in fundamental physics that may be answerable by means of atoms and molecules confined in traps. Can such a system be used to test the randomness of the physical processes such as the decay of the

excited state of an atom?

In principle, it might be possible to carry out such a study by means of a single atom or ion that is suitably trapped in a potential well, using some kind of radiation source to excite it and a suitable photomultiplier to detect the photons arising from radiative decay. But the photons used for excitation would have precisely the same frequency as those to be detected by the photomultiplier. If the average lifetime of the excited atom were to be large enough for the intervals between excitation and decay to be measurable, the excitation would be only weakly coupled to the exciting radiation, which would enhance the likelihood that the photomultiplier would be more often activated by exciting radiation than by that from a genuine decay of an excited atom.

So Erber and Putterman propose a neat atomic switch, a way of telling whether a single atom is at any point excited into one of these comparatively long-lived states. To fit the need, the atom must have two excitation levels, one of which is short-lived and the other long-lived. By means of two lasers, one tuned to each of the excitation frequencies, it should be possible to be sure that the atom at any time is in one of three states, the unexcited ground state or one or other of the two excited states. But the amount of time for which the atom is in the short-lived state can be measured by recording the photons arising by the spontaneous fluorescent decay of that short-lived state. Moreover, because the frequency of the transitions in each direction will be high, if the disparity of lifetimes is great enough, when there is no short-lived fluorescence, the atom should be excited into the long-lived state. In other words, the experiment that naturally suggests itself is a means of measuring the length of time for which a single atom persists in the long-lived state.

Erber and Putterman go on to say that these lifetimes, which should be unaffected by any influence of the external observer, should be strictly random numbers. Paradoxically, they remark, the simplest of all physical systems, a single atom caught in a trap, should be more capable than all but the most complicated computing machinery of generating strictly random numbers. The more important point is that the system is yet another way of testing predictions of quantum mechanics. That is another challenge, but increasingly an academic one.

John Maddox

Developmental biology

Mice, mating types and molecular mechanisms of morphogenesis

from Miranda Robertson

Two meetings this year* have marked the arrival of a molecular biology of embryonic development. At both, much emphasis has been placed on the universality of developmental mechanisms in phylogeny. In what follows, I shall use a few selected examples to illustrate the parallels that are being drawn between mice, flies and microbes and to examine briefly what they may mean.

I should state at the outset of this article that the omission of *Drosophila* from its title is not merely a matter of assonance: the dazzling achievements of *Drosophila* molecular genetics have been extensively discussed in earlier articles in these pages and the most recent advances are soon to be the subject of another. I shall therefore mention them only in connection with the arresting discovery that the so-called homoeo-box sequences, comprising part of the homoeotic genes that control *Drosophila* morphogenesis, have highly conserved counterparts in vertebrates (see ref. 1). The hope has been that these sequence homologies may provide one of two new routes to the genes that control morphogenesis in mice and men. The second route that has opened up at much the same time is through the transgenic mouse. DNA microinjected into a mouse embryo will occasionally become integrated into its chromosomes in such a way as to disrupt the normal process of development and thus in principle act as a traceable marker for a gene involved in its control. The work reported by Woychik *et al.* in this issue of *Nature*² has already been publicized³ as a success for the second approach: in a series of experiments in Philip Leder's laboratory in which the *c-myc* gene was injected into mouse embryos, on one occasion the gene fortuitously became integrated so as to produce a recessively inherited deformity of the limbs.

How far can the deformed mouse be regarded as a mammalian equivalent to the homoeotic mutants of *Drosophila*? And how likely is it that the mammalian homoeo boxes will provide a direct route to genes essential for morphogenesis in mammals?

Mice

The homoeo boxes of *Drosophila* are coding sequences of DNA, usually about 180 base pairs in length, first discovered at the 3'-end of the homoeotic genes, mutations in which cause the transformation of one

body part into another. Homologous sequences are found in worms, frogs, mice and man — and in yeast, which seems an embarrassment to their putative role in metazoan morphogenesis (but see later).

Drosophila homoeo boxes fall into two classes (1 and 2), which are about 50 per cent homologous in sequence. But the homology between the homoeo boxes of each class across species is about 80 per cent. At the time of its discovery this was, and is still, the most compelling evidence that the sequence homologies between the homoeo boxes of fruitflies and vertebrates have any functional significance. Other kinds of evidence are somewhat thin. It is now clear that these sequences are expressed in vertebrate embryos, and that their expression is in some cases temporally regulated and tissue specific. Class 1 sequences, for example, are expressed from day 13 in mouse, principally in the dorsal neural tube (Frank Ruddle, Yale); Gail Martin (University of California, San Francisco) finds expression of both class 1 and class 2 sequences between about the ninth and seventeenth days of gestation, and in embryonal carcinoma cells of mouse⁴ and man (Robert Tjian, University of California, Berkeley) class 1 sequences are expressed only after the induction of differentiation.

There is, however, nothing to link any of this with morphogenesis. Nor do mouse genetics provide evidence of anything that might be a homoeotic mutation. There is a morphogenetic mutant (*Tail-short*) that maps near the class 1 homoeo-box cluster on chromosome 11 (see ref. 1); but the association may be entirely adventitious. The mutation induced by *c-myc* in the transgenic mice of Woychik *et al.* may prove more informative.

The principal feature of the limb deformity in the mutant mice is the fusion of some of the distal bones of the limbs, and Leder and his colleagues have been able to show, through genetic crosses, that the mutation is allelic to a natural recessive mouse mutation, *ld*, that also causes bone fusion and maps, like the inserted *myc* gene, to chromosome 2. There is, moreover, a naturally occurring dominant mutation, *1st*, that maps to roughly the same region and causes the reverse phenotype — bone duplication. The dominant mutation has not yet been shown to be allelic to the recessive; but if it is, the two mutations may reflect, respectively, an excess and a deficiency of a morphogen or growth factor⁵. In principle, then, analysis of the site of the *myc* insertion, which has already been cloned,

should yield the intact gene encoding the factor, and the first identification of an embryonic morphogen.

In practice, however, it has not yet been possible to identify the inactivated gene and Leder's optimism is somewhat guarded, as was the response to his presentation at Cold Spring Harbor. Morphogenetic mutants are not always interesting, as experience with invertebrates has shown. There is for example a *Drosophila* wing-deformity mutant, *rudimentary*, that is simply the consequence of a metabolic deficiency affecting pyrimidine synthesis; and it was repeatedly pointed out that quite non-specific perturbations may disrupt development in an apparently systematic way (this has been a problem, for example, with attempts to apply to vertebrates the technique of specific inhibition of gene expression with anti-sense RNA). It seems unlikely that the fruitfly fraternity will be impressed by mouse geneticists until they can produce a mutant with a perfectly formed paw whose only abnormality is that it ought to have been an ear.

Mating types and mechanisms

In the absence of any evidence of a role for the vertebrate homoeo boxes in the genetic control of development, what might the evolutionary conservation of the homoeo boxes signify? An answer may be suggested by the ostensibly surprising discovery of homologous sequences in yeast — though it is only fair to say that the homology between yeast and metazoan homoeo boxes is of an altogether lower order than those between the metazoan sequences.

It is believed that *Drosophila* homoeo boxes encode protein domains that bind specifically to DNA. It is therefore probably significant that the yeast sequences homologous to the *Drosophila* homoeo box occur in the gene encoding $\alpha 2$ protein, a regulatory molecule that coordinates the expression of genes determining the mating type of yeast cells by binding to a specific sequence in their flanking DNA⁶. It has long been the guiding principle of Ira Herskowitz's laboratory, in which most of the investigations of the $\alpha 2$ protein have been performed, that the control of mating type in the single-celled yeast embodies the fundamental principles of differentiation in higher animal cells. Moreover, yeast mating type has one striking advantage over fruitfly homoeosis: while nothing is yet known about the molecular basis for the homoeotic phenotype, a good deal is now known about the molecular mechanisms that underlie the specification of yeast cell type.

The yeast *Saccharomyces cerevisiae* has two haploid mating types, *a* and α , each of which releases a factor that binds to a specific receptor on cells of the opposite mating type. This causes the two cells to fuse to form an *a*/ α diploid. In *a* cells, the genes specifying the α factor and the *a*-

*The Cold Spring Harbor Symposium on Molecular Biology of Development, 29 May–5 June 1985, and *Nature's* Conference on Genes and Systems in Development, San Francisco, 6–9 October 1985.

factor receptors are silent, whereas those specifying the a factor and the α -factor receptor are active; the converse is the case in α cells; and in a/α cells, both sets of mating-type genes are silent and those that control sporulation, which are silent in the haploids, are activated. Mating type is controlled by a single locus, the mating-type locus *MAT*, which contains an a allele in a cells and an α allele in α cells. The gene products of these alleles are regulatory proteins. Herskowitz and his colleagues have been able to show that $\alpha 2$, one of two products of the α allele, binds to a specific sequence upstream of the a -specific genes and represses them (refs 5 and 6, and Kathy Wilson, University of California, San Diego). The other product, $\alpha 1$ activates the α -specific genes. In a cells, the α -specific genes are silent because of the absence of $\alpha 1$, and the a -specific genes are active because of the absence of $\alpha 2$.

In a/α cells, a completely new pattern of gene expression is generated by the combination of regulatory proteins from both *MAT* alleles. The most recent evidence from Herskowitz's laboratory suggest that the sporulation genes are activated in the diploid cell by repression of *RME1*, a gene whose product inhibits sporulation genes in haploids. The repression of *RME1* requires $\alpha 2$ and $a 1$, which is a product of the *MAT a* allele. Although its mechanism is unknown, the DNA-binding properties of $\alpha 2$ make it likely that an $\alpha 2$ - $a 1$ complex binds, perhaps cooperatively, to a regulatory site in the *RME1*-flanking DNA. Thus the combination of two regulatory proteins may generate a novel specificity not possessed by either alone. This proposition has been

strengthened by the identification of a presumptive $a 1$ - $\alpha 2$ binding site whose sequence is related to the known $\alpha 2$ binding site⁷.

Back to mice

How does the molecular mechanism of mating-type determination in yeast help with the two questions with which I began: what is the significance of the homoeo-box homologies, and can transgenic mice provide a mammalian alternative to the homoeotic mutants of fruit-flies? It is not known whether the homoeo-box encoded domain of $\alpha 2$ is directly involved in the binding of DNA, but it is known that mutations in this region abrogate its activity. Since its activity has been shown⁸ to depend upon the formation of $\alpha 2$ multimers as well as on binding to DNA, the homoeo-box encoded domain may be responsible either for binding DNA or for binding between the protein monomers.

It follows that if the conserved homoeo-box sequences do, as seems likely, reflect conserved functions, then it is their binding specificity that is being conserved; but it does not necessarily follow that this binding — whether to DNA or to other proteins — has anything to do with the control of development. There is no dearth of evolutionary precedents for the use of the same highly conserved structure in different physiological pathways: yeast and mammalian *ras* have recently turned out to be a case in point (see ref. 8) and it is a commonplace that a highly conserved hormone may act through a highly conserved receptor to produce a wide variety of unrelated effects in different cells.

What the yeast data may illuminate are the principles on which gene regulation

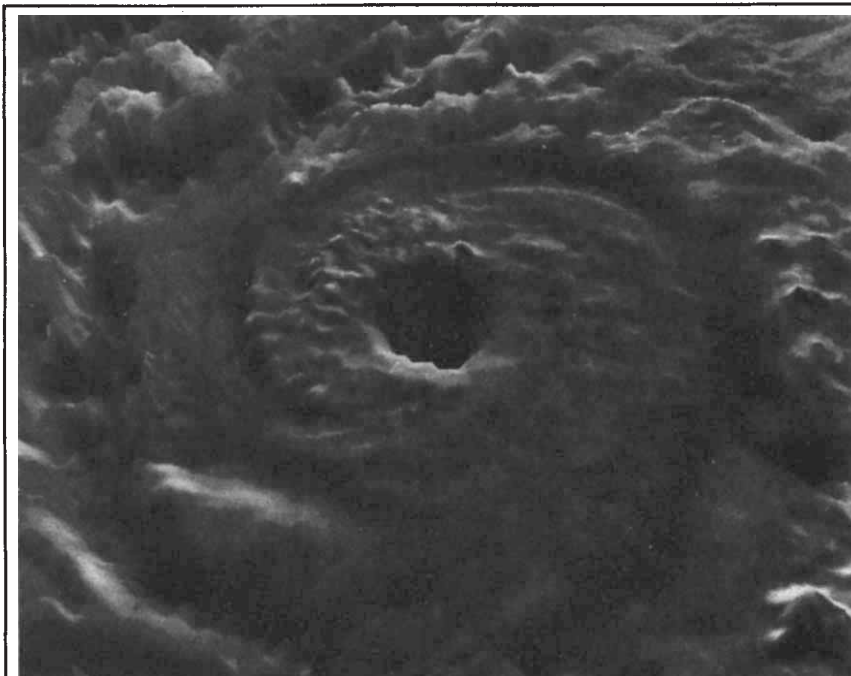
may work during embryogenesis, rather than the actual proteins through which a particular pattern of regulation is brought about. For example, it is generally accepted that differential gene regulation during development must be controlled in part by signals between cells. Thus it must depend on the temporally and spatially patterned expression by developing cells of the genes encoding the signals and their receptors. Some patterning of this sort is present in microcosm in yeast: in some strains, the a and α mating types (and thus the two factors and their receptors) are expressed in alternate haploid generations, but with the complication that only mother cells may make the switch. The mechanism of this patterned switching is not fully understood but analogous mechanisms may underline patterned developmental switching in higher organisms.

And while changes in gene expression in embryos are not likely to be brought about by the fusion of cells of two different types, it is extremely likely that differentiation is the consequence of varying combinations of cooperating regulatory proteins, as with $a 1$ and $\alpha 2$ of yeast (induction of a new regulatory gene by an extracellular signal could take the place of the fusion of haploid cells in producing new combinations). As it happens, a transgenic mouse recently reported in *Nature*⁹ may provide an approach to the question of novel regulatory combinations in mammalian development. Swanson *et al.* have produced a mouse containing the growth-hormone gene fused to the metallothionein promoter and expressing the fusion gene in selected subsets of neurones in the brain, where neither growth hormone nor metallothionein is normally expressed.

It is traditional in development biology to distinguish between differentiation and morphogenesis. Under this distinction, it is differentiation that is disrupted in the Swanson *et al.* transgenic mouse, and morphogenesis in the Woychik *et al.* mouse. At the molecular level, however, differentiation and morphogenesis have the same mechanism, morphogenesis simply being a consequence of differential expression of genes that control developmental switches. It is the power of the yeast system to show how differential gene expression at a master regulatory locus, such as *MAT*, may give rise to patterns of differential gene expression of the sort that must underlie morphogenesis. □

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Miranda Robertson is Biology Editor of *Nature*.



Hurricane Allen on 8 August 1980 when its eye was above the Gulf of Mexico, as shown by application of a new computer imaging technique to meteorological data (Hasler, A.F., Pierce, H., Morris, K.R. & Dodge, J. *Bulletin of the American Meteorological Society* **66**, 798; 1985).

Angiogenesis

Isolation of a protein that stimulates blood vessel growth

from Lance Liotta

A TEN-YEAR scientific quest to characterize a substance that can stimulate angiogenesis — the growth of new blood vessels — has ended with a triplet of papers in which Bert Vallee and colleagues describe the purification of a protein they call angiogenin and the cloning of its gene^{1,3}. The quest was based on the pioneering work of Judah Folkman who demonstrated that tumour growth requires nourishment by the continual ingrowth of new blood vessels, and suggested that this is the result of a diffusible substance that he termed tumour angiogenesis factor (TAF)^{4,5}. Recognizing the therapeutic potential of an angiogenesis inhibitor, the Monsanto Company provided a \$23 million endowment to Harvard University in 1976 to support Folkman with Vallee, who was to contribute the biochemical expertise necessary for rigorous protein purification. About five years into the research, Vallee and Folkman split their research into independent directions. As a result, Folkman is not associated with the papers on angiogenin, which Vallee considers to be different from TAF.

Folkman and Vallee have used different approaches to the purification of angiogenic substances and this has led to the discovery of two types of purified substance. One is exemplified by a heparin-binding growth factor identified by Folkman's group. Folkman reasoned that angiogenesis begins with the migration and growth of capillary endothelial cells. His group therefore developed the first cloned cell lines of capillary endothelium, and *in vitro* assays to identify and purify factors that are mitogenic for endothelial cells. Klagsbrun and Shing in Folkman's laboratory purified a heparin-binding endothelial mitogen which induces new vessel formation when injected into the rabbit cornea⁶. Subsequently, several groups purified heparin-binding endothelial cell mitogens that are angiogenic⁷⁻⁹. A recent example is the report by Gospodarowicz's group⁷ of two polypeptide mitogens for endothelial cells. The primary structure of one of them — bovine pituitary basic fibroblast growth factor (FGF) — was determined and compared with the amino-terminal sequence of the other — brain acidic FGF. The purified basic FGF is a potent angiogenic substance in chick chorioallantoic membrane (CAM) assays.

Instead of using the endothelial cell as a starting point for purification, Vallee's group has accumulated and used large quantities (2,000 litres) of conditioned medium from human colon carcinoma cultures. The proteins in the medium were

fractionated by cation exchange and reverse phase liquid chromatography, and directly assayed for angiogenesis in the CAM assay. Purified angiogenin has a relative molecular mass of 14,000 and does not bind heparin. Only 3.5 pmol are required to induce blood vessels in the cornea by a mechanism that is apparently unrelated to endothelial migration or proliferation.

It is widely believed that angiogenin and the heparin-binding proteins may be members of a whole family of factors that induce and regulate the growth of new blood vessels; the angiogenesis literature contains many reports concerning a variety of different factors at various stages of purity, all of which can induce vessel growth in the cornea. From one point of view this is expected, since the development of a new capillary network is a complex process involving a number of steps. One of the first steps may be dissolution of the subendothelial basement membrane at the point of endothelial cell outgrowth¹⁰. Subsequent steps include endothelial locomotion, endothelial tube formation, development of capillary loops, renewed establishment of the basement membrane and maturation of the vascular channels¹¹. These steps may be coordinated by multiple signals from the endothelium and other host cells in response to the angiogenic stimulus. Angiogenesis may be analogous to the blood coagulation cascade in that one event leads to the next through the action of specific mediators. Although

angiogenin is not the only substance that can induce angiogenesis, it may be a key trigger for the process.

The most significant accomplishment of Vallee's group is the cloning of the angiogenin gene, which turns out to be one of the small number of mammalian genes known that lacks introns. The amino-acid sequence of angiogenin predicted from the gene sequence is strikingly homologous to human ribonuclease, including conservation of the amino-acid residues that are essential for the activity of ribonuclease. The physiological significance of the homology is unclear, since angiogenin lacks ribonuclease activity and ribonuclease itself is not angiogenic. Expression of the gene for angiogenin is probably not limited to tumour cells. Nevertheless, angiogenesis may be more tightly regulated in normal tissues than in neoplastic tissues.

The cloning of the gene for angiogenin now makes it possible to study one potential mode of regulation of angiogenesis. The gene can also be used to produce large quantities of angiogenin so that its mechanism of action can be fully studied. This may ultimately lead to pharmacological strategies for inhibiting or augmenting the growth of blood vessels. Control of angiogenesis could have an impact on human diseases ranging from neoplasia to myocardial ischaemia. □

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Lance A. Liotta is Chief of the Laboratory of Pathology, National Cancer Institute, Bethesda, Maryland 20205, USA.

Palaeontology

Fossil radiography

from Simon Conway Morris

MUSEUM visitors will be familiar with the striking brassy-yellow colouration of many ammonites, the result of replacement of the fossil by iron pyrites (FeS₂). Not surprisingly, this replacement typically involves the hard skeletal parts that provide the stock-in-trade of most palaeontologists. There are, however, extraordinary instances where pyritization may preserve the soft parts and more lightly skeletalized components of animals and plants from the otherwise inexorable processes of decay (Fig. 1). The Lower Devonian Hunsrück Slate (about 400 Myr) of West Germany is widely recognized as one of the most important deposits for soft-part preservation in the fossil record, and on page 53 of this issue

Wilhelm Stürmer describes another major find from this palaeontological gold-mine in the form of a unique specimen of a squid, aptly named *Eoteuthis*.

Stürmer's description is important on several counts. Not only does this discovery extend significantly the geological timescale of the teuthids, but it may ultimately result in a revision of current notions about cephalopod phylogeny, especially for the coleoids (squid, cuttlefish and octopus). At present, because coleoids generally lack preservable hard-parts, there is a lamentable fossil record that is almost entirely derived from Jurassic or younger rocks, and there is little consensus concerning the phylogenetic relationships within the squids and their

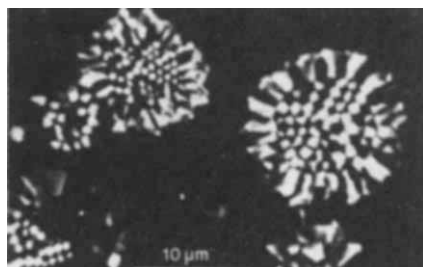


Fig. 1 A back-scattered electron image obtained with a scanning electron microscope of pyrite framboids from exceptionally well preserved trilobites with soft parts, from the Beecher's Trilobite Bed, New York State.

relatives.

Eoteuthis is one more addition to the roster of remarkably diverse finds from the Hunsrück, which boasts a wealth of otherwise poorly known forms. Among others, there are trilobites with preserved appendages, pycnogonids and a variety of more enigmatic arthropods, ctenophores, polychaetes, nautiloids, starfish and crinoids (Stürmer, W., Schaarschmidt, F. and Mittmeyer, H.-G., *Kleine Senckenberg-Reihe* 11; 1980). This wealth of fossils, providing the most complete insight into a mid-Palaeozoic marine community, continues to be augmented both by amateur collectors and from Stürmer's private quarry in Kaisergrube.

Stürmer uses sophisticated X-ray equipment and high-resolution photographic materials to produce radiographs of pyritized fossils concealed in the slate (Fig. 2). The density of the pyrite in contrast to the surrounding shale makes radiography particularly suitable, especially if the specimen is largely concealed with the rock. By carefully controlling the conditions of exposure and using image enhancement, formerly obscure structures may be revealed to a resolution of at least 5 μm . His recent descriptions of the Hunsrück biota and of material from other special palaeontological sites (Stürmer,

W. *Interdisc. Sci. Rev.* 9, 1; 1984) highlight the success of the techniques. They contrast with traditional methods of preparing Hunsrück fossils by mechanical excavation that require utmost care to avoid damaging the gossamer films of pyrite.

The precise conditions governing pyritization in the Hunsrück are still obscure. The preservation of soft-parts, probably by filling cavities or forming coatings, by pyrite precursors such as greigite (Fe_3S_4), probably took place very shortly after burial. This would accord with recent textural and sulphur isotope studies of pyrite paragenesis (Hudson, J.D. *Sedimentology* 29, 639; 1982 and Love, L.G., Coleman, M.L. & Curtis, C.D. *Trans. R. Soc. Edinb. Earth Sci.* 74, 165; 1983), and suggests that this early stage of pyritization could persist only as long as bacterially

mediated sulphate reduction occurred. Interruption of sulphate influx from the overlying seawater, perhaps by rapid sediment deposition, could have arrested the bacterial activity that was presumably contributing to the overall biodegradation. But this hardly explains the selective replacement of the Hunsrück organisms, or the scarcity of similar fossil occurrences. Another example is the celebrated Beecher's Trilobite Bed in the Upper Ordovician of New York (Cisne, J.N. *Palaeontograph. Am.* 9 (53); 1981). Perhaps the rarity is more apparent than real and surveys of other fine-grained sediments will yield some surprises. $\square$

Simon Conway Morris is in the Department of Earth Sciences, University of Cambridge, Cambridge CB2 3EQ, UK.

Nuclear physics

Pions still pose problems

from Philip J. Siemens

A REPORT¹ that pi mesons, or pions, have been produced in collisions of complex atomic nuclei accelerated to only 23 per cent of the speed of light has added to the problems of nuclear physicists trying to explain pion production in low velocity nuclear collisions. Simple predictions based on the idea that pions result from collisions between individual pairs of neutrons or protons fail to account for pion production at such low collision velocities and the hope is that the phenomenon will reveal new details of nuclear structure.

Since each nucleus will normally exist in its state of lowest energy, a pion cannot be removed without supplying sufficient energy to provide not only for the pion's kinetic energy of motion, but also for the energy mc^2 associated with its mass m . The same amount of energy would also be sufficient to create a new pion that had not been part of the original nucleus.

The more usual way of liberating pions from nuclei (or creating them — the two processes cannot be distinguished) is to bombard nuclei with accelerated projectiles such as neutrons or protons; the kinetic energy of the accelerated particle produces or liberates a pion when it strikes the nucleus. Starting with a knowledge of how pions are produced in collisions between individual neutrons or protons, theorists have been able successfully to predict features of the production of pions from complex nuclei, such as how often they are produced and their distribution in energy and angle. These predictions are based on the idea that a pion is created when the projectile — which may be a photon, electron or pion, as well as a neutron or proton — strikes one of the neutrons or protons in the target nucleus; the resulting pion may either escape or be reabsorbed by the nucleus.

The difficulties start when complex nuclei are used for projectiles as well as for targets. In the past few years, new accelerators have been commissioned that are able to accelerate complex nuclei, such as carbon or neon, to speeds where the total kinetic energy of the nucleus is sufficient to create a pion, whereas the kinetic energies of the individual neutrons and protons are too small for the purpose. Although pions are seldom produced in these collisions — as rarely as once in a hundred million collisions — they have been observed, initially in 1979 at the Lawrence Laboratory in Berkeley, California². Surprisingly, the pions were produced from beams of nuclei with a velocity of 0.43 c , whereas the minimum velocity necessary to produce a pion from a neutron or proton striking a target of protons is 0.64 c . In 1982, an experiment³ at CERN in Geneva produced pions from nuclear beams with a velocity of 0.4 c and the following year⁴ the National Superconducting Cyclotron Laboratory in East Lansing, Michigan yielded pions from beams of a velocity of only 0.27 c . This downward trend is continued by the 0.23 c reported by Oak Ridge National Laboratory in Tennessee⁵. Experiments in progress at accelerators at Caen, France and at Texas A&M University in the United States may continue the trend.

Because the pions observed in these experiments cannot be produced by collisions of individual pairs of neutrons or protons, they ought to reveal information about the structure of the nucleus as a whole. Two theoretical models have been partially successful in interpreting the observations. The first model, by Jorg Aichelin and George Bertsch of Michigan State University⁶, explains the number of pions by assuming that the projectile and

IMAGE
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REASONS

Fig. 2 Radiograph of a fossil crinoid from the Hunsrück Slate. Also known as sea lilies, crinoids are echinoderms, related to starfish and sea urchins. Only two specimens of this species have been found and this picture has been assembled from radiographs of several species. (Photograph courtesy of W. Stürmer).

target fuse together, distributing the projectile's kinetic energy throughout the combined nucleus in the form of heat. Applying statistical mechanics, this model gives a good account of the pions' energies for the faster beams but cannot explain why their average kinetic energy does not decrease as the beam velocity falls below 0.35 *c*. Below this speed, the pions' kinetic energy distribution seems to retain a high temperature, even though the excited nuclei that emit them are cooler. The model also makes no prediction about the angles at which the pions are emitted.

To explain the angular distributions of the pions requires quantum mechanics, because the wavelength of their matter waves is larger than the size of the nucleus that emits them. So far, the only applicable quantum theory has been developed by Walter Greiner, Berndt Mueller and co-workers at the University of Frankfurt. This model draws on an analogy with the emission of electromagnetic waves: the deceleration of the nuclei as they collide provides a time-varying current which causes radiation of pionic matter-waves. This theory gives a reasonable account of the angular distribution⁶ but predicts —

contrary to experiment — that no pions will be emitted in collisions of carbon beams with carbon targets, since carbon has zero isotopic charge (the pionic analogue of the electric charge). An *ad hoc* modification of the theory would permit a reasonable reproduction of the data but would fail at still lower energies, where it would continue to predict pion emission even when the total energy is insufficient.

The theorists' inability to understand the cooperative mechanism of pion production in low-velocity nuclear collisions has not discouraged the experimenters from competing to lower the velocity even further. Since pions are the main agents of the strong forces that hold nuclei together, theorists hope that the data will eventually provide a better understanding of nuclear forces. □

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Philip J. Siemens is Professor of Physics at Texas A&M University, Texas 77843, USA.

Geology

East Asian tectonic collage

from A.M.C. Şengör

As the accompanying map shows, Asia consists of numerous continental and oceanic fragments stitched together by continental collisions. These not only enlarged the continent, but also disrupted parts of it through collision-related rifting and strike-slip faulting, as may be seen in the current convergence between India and Eurasia¹. During the last decade China has emerged as an ideal place to study the processes of continental collision and evolution of orogenic collages²⁻⁶, offering a tremendous variety of collisional mountain belts that are now in, or were fossilized at, different stages of their evolution. Two papers in a recent issue of *Nature*, by Mattauer *et al.*⁷ and Peltzer *et al.*⁸ deal with the tectonics of one of China's most important mountain ranges, the Qin-Ling Shan — "the backbone of China proper".

Since the time of the expeditions of P. Armand David in 1866, Count Bela Széchenyi from 1877 to 1880, and especially Baron Ferdinand von Richthofen from 1868 to 1872, the Qin-Ling Shan has attracted the attention of geologists. The unravelling of how and when eastern Asia was assembled is critically dependent on the tectonic evolution and timing of collisions along this belt, but even the establishment of the age of the main deformation has been no easy matter. Mainly on the basis of von Richthofen's observations, Suess¹⁰ described it as a orogenic belt of medial Carboniferous age, whilst

Argand¹¹ saw in it a 'Hercynian chain' with pre-Devonian enclaves. In the first detailed study of the chain in 1929, Chai and Huang⁹ revised von Richthofen's stratigraphy and moved the age of the main orogeny into the Jurassic. Later, in his now-classic 1945 memoir¹², T.K. Huang pointed out that recent research had established the age of the Qin-Ling Shan as "pre-Jurassic". Although Huang left open the possibility of a late Palaeozoic ('Hercynian' as he called it) deformation, he strongly favoured an Indonesian (late Triassic) orogeny. During the last two decades, opinions on the age of the main orogeny along the Qin-Ling Shan have varied from Devonian (400 Myr) to late Triassic (210 Myr), with the majority of the Chinese and foreign authors favouring an Indonesian orogen with a polycyclic history stretching through the 'Caledonian' and 'Hercynian' cycles¹³⁻¹⁸.

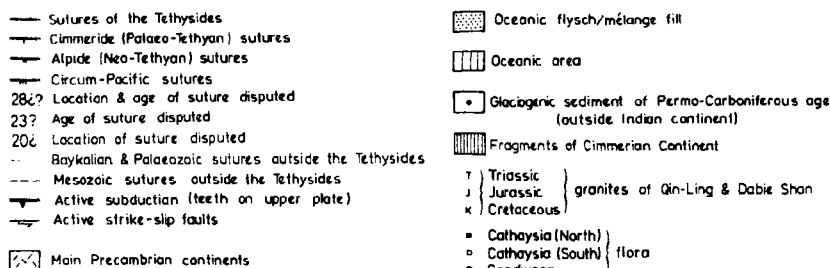
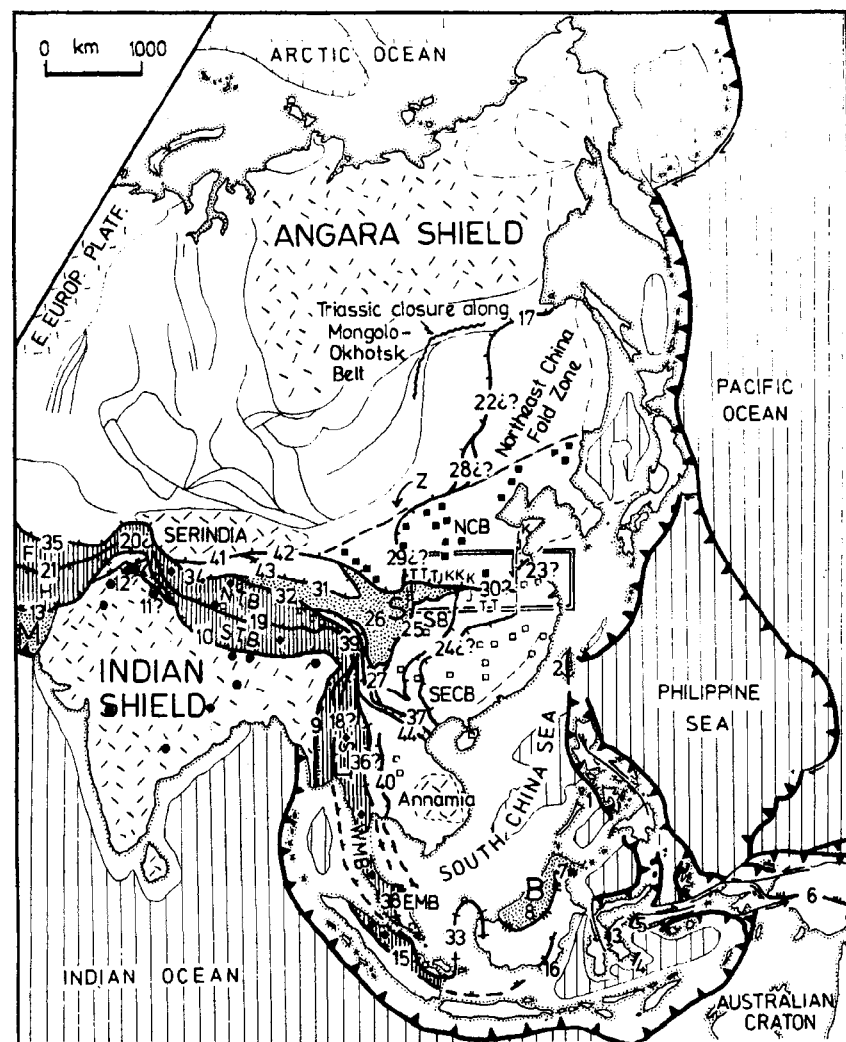
Mattauer *et al.*⁷ divide the Qin-Ling Shan into five longitudinal zones characterized by distinct rock assemblages and separated by major steep faults. In zones 1 through 4 (from north to south) they recognize a 'Caledonian' collision belt (an unfortunate feature of the paper by Mattauer *et al.* is the employment of obsolete and misleading Stellerian phase nomenclature that they seem to have inherited from their Chinese sources), and in the most southerly zone 5 they see an Indonesian intracontinental fold and thrust belt. They

also recognize a 'Hercynian' deformation and metamorphism in zones 2 through 4, that are also viewed as intracontinental, that is, post-collisional.

The implied view that the North and South China block had already collided by the Devonian represents a minority opinion even among Chinese geologists and conflicts with other evidence from palaeobiogeography¹⁹ (see figure) and palaeomagnetism^{20,21}. In my view, the geological map provided by Mattauer *et al.*⁷ does not contain cartographic evidence for a Devonian or even a late Palaeozoic collision along the Qin-Ling mountains (for instance, there is apparently no unconformable molasse of appropriate age nor any intrusion earlier than late Palaeozoic cutting the structures discordantly). Because the available evidence in the published literature overwhelmingly favours a Triassic collision^{4,13-20}, I obtained an independent opinion from K.J. Hsü, who had visited the same area as Mattauer *et al.* earlier this year under the guidance of Chinese geologists.

Hsü believes that the Qin-Ling Shan could be interpreted as a collision-type mountain belt, comparable to the Alps, that culminated in late Triassic time ('Indonesian'). He thinks that the stratigraphic evidence indicates only one orogenic cycle (Wilson cycle) stretching from the late Proterozoic to the late Triassic. Zone 1 of Mattauer *et al.* is viewed by Hsü as an Austroalpine-type rigid basement nappe, whose main internal deformation had already occurred probably in late Proterozoic time. In zone 2, Hsü sees Pennine-type folded nappes of basement material, whereas in the 'Central Qin-Ling Dome' (zone 3) an equivalent to the *schistes lustrés* and ophiolites is seen. Hsü, like Mattauer *et al.*, interprets this as the remnants of a former ocean. He believes the so-called 'Devonian trough' (zone 4) was a region of turbidite sedimentation, but disputes the Devonian age assigned to the turbidites here, for that age comes from macrofossils in exotic limestone blocks found in the turbidites. Hsü points out that Triassic ammonites have been recovered from similar rocks in the same zone farther west, thus making at least some parts of the turbidite sequence of the 'Devonian trough' Triassic.

The Triassic to early Cretaceous age of the main linear granite belt north of the Qin-Ling Shan, the 'Variscan' age of the earliest known subduction-related magmatism ('Variscan' or 'Hercynian' in Asia may in places reach well into the Triassic, emphasizing once more the misleading consequences of the employment of these terms outside their European type areas), and the pronounced difference both in the palaeophytogeographical and in the palaeomagnetic²¹ signature of the Permo-Carboniferous rocks on both sides of the Qin-Ling Shan, all seem to converge to support a Triassic collision. This becomes



Tectonic map of Asia showing the Phanerozoic sutures; Tethyside sutures, of which the Qin-Ling Shan (boxed area) is a part, are emphasized. The map also shows the distribution of late Palaeozoic (Permo-Carboniferous) glaciogenic sediment and flora distribution (except the Angara flora) to identify independent blocks of that age and their possible palaeobiogeographic affiliations. Notice how sharply the Qin-Ling suture separates the North Cathaysia realm from the South Cathaysia realm. Also notice the pronounced Triassic to Cretaceous batholith belt immediately north of the Qin-Ling suture.

Numbers refer to sutures: (1) Mindoro; (2) Taiwan; (3) Poso; (4) Butung; (5) Peleng; (6) New Guinea/Papua; (7) Sabah; (8) Sibiu; (9) Burma; (10) Indus-Yarlung Zangbo; (11) Shiok; (12) South Ladakh; (13) Waziristan; (14) Makran; (15) Sumatran; (16) Meratus; (17) Shilka; (18) Sittang Valley-Myitkyina; (19) Banggong Co-Nu Jiang; (20) Rushan-Pshart; (21) Waşer (Farah-Rud); (22) Da Hinggan Ling; (23) Huang Shan; (24) Central South China; (25) Longmen Shan; (26) Litang; (27) Jinsha Jiang; (28) Inner Mongolian; (29) West Ordos; (30) Qin-Ling; (31) Anyemaqen Shan; (32) Hoh Xil Shan; (33) Natuna; (34) Suture of the southern synclinorium of the western Kuen-Lun; (35) Herat; (36) West Thailand (Nan-Uttaradit); (37) Song Da; (38) Medial Malaya (Bentong-Raub); (39) Lancan Jiang; (40) Petchabun; (41) Suture of the northern synclinorium of the western Kuen-Lun; (42) Qilian Shan; (43) Qimantag; (44) Song Ma. Lettering: (B) Borneo accretionary complex; (EMB) East Malaya Block; (M) Makran accretionary complex; (NCB) North China Block; (NTB) North Tibetan Block; (S) Songpan-Ganzi accretionary complex; (SB) Sichuan Block; (SECB) Southeast China Block (SB+SECB=South China Block); (Z) Li Chunyu *et al.*'s North China suture (ref. 14). The map is compiled from data in refs 4 and 19.

the more plausible when one follows the suture both westwards into the Kuen Lun and eastwards into Korea, where it continues into the orogens of Triassic age^{4,14-16}.

A very significant point made by Mattauer *et al.*⁷ and Peltzer *et al.*⁸ is the presence of significant strike-slip motion along the Qin-Ling Shan in both Tertiary-Quaternary time and probably also earlier. The post-Eocene movement, of the order of several hundred kilometres, is rightly ascribed to the effects of the Himalayan collision. But the possibility of a few thousands of kilometres of motion during the Mesozoic (see ref. 20) presents acute problems in attempting palaeogeographic and palaeotectonic reconstructions. Thus, the isotopic or palaeontological ages obtained from slivers, or even entire terranes, dragged along such giant strike-slip systems may have no relevance to the orogenic history of the edifice in which they now lie embedded.

The two papers by Mattauer *et al.* and Peltzer *et al.* clearly indicate, in the light of the above discussion, that the Qin-Ling Shan has had an extremely complicated, multi-stage evolution, during which its constituent elements may have been repeatedly redistributed by several episodes of important strike-slip motion along the belt. This alone may help us to see why the interpretation of the tectonic history of this orogen is so difficult and prone to controversy. This must be true, albeit in different degrees and in different combinations, along all of the suture zones that hold together the Asian edifice, showing²² how dangerous and misleading it must be to consider orogenic evolution along cross sections alone. Much more work remains to be done before even a glimpse is obtained of the problems that are involved in understanding the orogenic evolution of the Qin-Ling Shan, as well as the assembly and disruption of Asia. □

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A.M.C. Şengör is in the Geology Department, Faculty of Mines, Istanbul Technical University, Istanbul, Turkey.

Cosmology

Radio supernovae as probes for Hubble's constant

from Alan P. Marscher

IT SEEMS remarkable that, over half a century after Edwin Hubble discovered that all distant galaxies are receding from us with velocities proportional to their distances, astronomers still cannot agree on the value of Hubble's constant, the slope of this relationship. This important parameter corresponds to the expansion rate of the Universe in most cosmological models, and can be used to determine the present age of the Universe in the standard big bang model. On page 25 of this issue, a new method, which utilizes the observed expansion rate of radio-bright supernovae to estimate Hubble's constant, is reported by Bartel and his colleagues¹. The method, while somewhat dependent on a model, obviates the systematic uncertainties inherent in optical distance determinations.

Using optical distance indicators, Sandage and Tammann² arrived at a value $H_0 \approx 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ for Hubble's constant, with an estimated upper limit of about 60. On the other hand, de Vaucouleurs³ and Bothun and colleagues⁴ obtained values between 90 and 100, with a lower limit of about 85. That these two limits are so incompatible illustrates the importance of systematic errors in the derivation of H_0 (see ref. 2 for an excellent summary). Nearly all critical distance determinations depend on the use of very few distance indicators (such as Cepheid variables) to obtain the distances to more remote galaxies. Therefore, uncertainties in the use of this small number of primary distance indicators become systematic errors in the determination of H_0 . What is clearly needed is a method independent of the derived distances of local group galaxies.

Fritz Zwicky maintained that supernovae were the only worthwhile extragalactic distance indicators. Whether or not this proves to be the case, supernovae

at least have the advantage that their luminosities are high enough to allow detection at distances greater than 1,000 Mpc. It is also well established that they represent exploding stars with expansion velocities that can be measured via the Doppler shifts of the emission and absorption lines in the optical spectrum. One method for calculating the distance to a supernova, attributed to W. Baade and A. Weselink, uses the observed expansion velocity and the assumption that the optical-infrared continuum represents black-body emission from an opaque, expanding sphere of gas. The shape of the continuum yields the temperature of the black body which, together with the radius calculated by multiplying the expansion velocity by the time since the onset of the explosion, allows one to calculate the luminosity. The relationship between this derived luminosity and the observed flux depends only on distance, which can then be determined. The main difficulty with this method is that the optical-infrared continuum does not fit a black-body curve precisely and the deviations must be modelled theoretically for the method to be applied successfully⁵.

Bartel *et al.*¹ use what at first glance appears to be a much more straightforward approach: by using centimetric-wavelength, very-long baseline interferometry (VLBI), they have been able to measure the angular diameter of the radio source associated with supernova 1979c in the galaxy M100 in the Virgo cluster. Since the true diameter of the supernova can be derived from the optically-determined expansion velocity, the distance to the supernova (and the galaxy containing it) can be readily calculated. Using this distance to M100, they derive a value of $H_0 = 65^{+45}_{-25} \text{ km s}^{-1} \text{ Mpc}^{-1}$. While this range of possible values hardly solves

the controversy over Hubble's constant, it is exciting that an attempt has been made to employ such an apparently straightforward technique.

The large uncertainty in the value of H_0 obtained by this method arises mainly from two distinct difficulties. The first is observational. Extragalactic supernovae are faint at radio wavelengths, so much so that only a handful have been detected by the most sensitive radio instruments available. The successful use of VLBI requires a strong detection by at least 3 or 4 antennas, but with the current VLBI configuration supernova 1979c is only weakly detected. The combination of the very-long baseline array (to be built in the United States by about 1990) and large European radio dishes should improve this situation substantially.

The theoretical difficulty is a little stickier. The optical expansion velocity is only relevant here if the radio emission is co-spatial with the optical spectral-line emission. In fact, the leading model⁶ for the radio light curves of supernovae places the radio emission in a shock wave outside the bulk of the debris from the explosion. Bartel *et al.*¹ assume that the radio brightness distribution lies somewhere between that of a uniform sphere and that of a ring with an expansion velocity closely related to that obtained from the optical spectra. It has been shown⁷ that these extreme cases lead to values of H_0 which bracket those obtained using more realistic brightness distributions. This uncertainty in the appropriate geometry leads to the wide range of values for H_0 allowed by the data of Bartel *et al.* Eventually, the actual radio-brightness distribution of a supernova might be measured directly using VLBI, obviating the need to rely on theory. Even then, frequency-dependent apparent diameters (possibly observed by Bartel *et al.*), and other complications, might limit the ultimate accuracy of the method.

If, as expected, further refinements and continued observation improve the accuracy of the new technique, we will be blessed with a cosmological probe that is independent of all the systematic uncertainties that haunt the current optical methods. Such a breakthrough would enable cosmologists to get on with their business of model making, released from the nagging fear that the size scale and age of the Universe they have adopted could be off by a factor of two. □

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Alan P. Marscher is Assistant Professor of Astronomy at Boston University, 725 Commonwealth Avenue, Boston, Massachusetts 02215, USA.

100 years ago

HYDROPHOBIA

ONCE MORE M. Pasteur is attracting the attention of the civilised world by his brilliant investigations . . . a boy of nine years old had been terribly worried on the 4th of last July; not only bitten in parts covered by his clothes but also on the hands. He was rescued covered with foam, and bleeding from no less than fourteen wounds. There was no question that the dog was mad, and in all human probability this child, Joseph Meister, was doomed to a certain and horrible death. M. Pasteur felt himself justified in applying the means to this suffering fellow creature, which had already proved efficacious in the case of brutes.

The inoculations were made with a subcutaneous needle, began on the 7th, and were

concluded on the 16th of July. "Control experiments" were made with the same injections upon rabbits, and proved that the virus was active. Moreover, since the effects of the modified virus, when introduced into an unprotected animal, are rapid and severe, and its period of incubation extremely short, the result of the attempt to rescue the child from a horrible death would soon be apparent. If he had died of hydrophobia, it would probably have been within a month. If he survived this period there was every reason to hope that he would be as much protected against its future manifestation as the dogs which had been tested before.

Joseph Meister was in perfect health at the end of August, at the end of September, and at the end of October. M. Pasteur believes that he is safe from hydrophobia for the rest of his life. From *Nature* **33** 1, 5 November 1885.

A new protein sequence data bank

SIR—A protein sequence data bank, called PGtrans, is available from our laboratory. This data bank is generated by automatic computer translation of the well-known nucleotide sequence library GenBank (established by Bolt, Beranek and Newman Inc. and Los Alamos National Laboratory). For this we designed a translator program which scans and interprets the specific information fields of each GenBank entry: DEFINITION, SOURCE and FEATURE table. The translator program can thus decide if the corresponding nucleotide sequence includes a coding region, and what is the proper genetic code to use to translate it into a putative protein sequence.

For each coding region successfully translated, the program creates a PGtrans entry including a text constituted of several fields: CODE, NAME, SOURCE, REFERENCE and COMMENT, followed by the amino-acid sequence. The translator program checks for unexpected 'stop' codons eventually found in coding regions. If a 'frame shift' allows the errored coding region to be rescued, the tentative amino-acid sequence is included in PGtrans. However, a warning is included into the COMMENT field to indicate a potential problem. The current PGtrans databank corresponding to GenBank (35.0) contains 3,107 entries for a total of 483,649 residues. Figure 1 shows the

entry-length distribution for PGtrans 29.0. A listing of the encounter anomalies is routinely communicated to the makers of GenBank.

The main purpose of PGtrans is to offer a direct access to all amino-acid sequences coded among GenBank nucleotide sequences and thus to be an efficient tool for protein homology searches. Its format is compatible with the rest of our sequence analysis software¹ and will be consistent with the recommendations of the CODATA task group on protein sequence data banks². Since we conserved the minimum of text information necessary to identify the protein, PGtrans is a compact data bank (1.4 Mbytes) which could fit for few more years into microcomputer environments. Among other efforts in gathering protein sequence data^{3,4}, the role of PGtrans is to provide a rapid update of new protein sequences determined by nucleic acid analysis. Magnetic tape copies (card image) of PGtrans will be distributed free of charge upon request to our laboratory.

JEAN-MICHEL CLAVERIE

ISABELLE SAUVAGET

Unité d'Informatique Scientifique,

Institut Pasteur,

75724 Paris Cedex 15,

France

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Three-dimensional electron lattice

SIR—*Nature* reported on 14 February (313, 527; 1985) the discovery of a three-dimensional electron lattice in *n*-HgCdTe by Rosenbaum *et al.* (*Phys. Rev. Lett.* **54**, 241; 1985). In fact the discovery was made in my laboratory in 1979. Our response to Rosenbaum *et al.* and relevant references can be found in *Physical Review Letters* for 22 July (55, 443; 1985).

G. NIMTZ

Universität zu Köln,
Physikalisches Institut,
Zulpicherstrasse 77,
5 Köln 41, FRG

Shadow boxing with Darwin

SIR—Dawkins' taunting review¹ of Eldredge's book *Time Frames: The rethinking of Darwinian evolution and the theory of punctuated equilibria* raised important issues of evolutionary theory that can only but invite comment.

There always have been two components to Eldredge and Gould's theory of punctuated equilibria, as the phrase suggests explicitly — long periods of stasis (equilibria) punctuated by relatively much shorter periods of change. To reiterate, yet again, that the periods of change potentially fall within traditional boundaries of gradual Darwinian evolution by means of natural selection, and to ignore the major periods of stasis (a device which allows Dawkins to dismiss the whole concept as an "interesting little theory . . . lying firmly within the neo-Darwinian synthesis") reduces scientific evaluation to a parlour game of one's own invention, which whilst entertaining and superficially coherent, does not offer proper scientific guidance for the general reader.

If we accept, for the moment, that stasis is a widespread observation in the geological record, and if too we accept that the forces behind selection are the only means for promoting biologically useful novelties throughout a population (setting aside contemporary knowledge that the molecular behaviour of the genes can effect similar changes), then it is surprising that little significant evolution occurs over periods of time that may span from hundreds of thousands to millions of generations, in a diverse range of species. The point is not that evolution is gradual "during the times that it is actually happening" (Dawkins' emphasis) but that it seemingly is not happening for most of the time. This was the message of the infamous Chicago meeting, which many of us took home, and which demands an explanation.

Attempts to bring stasis within the neo-Darwinian synthesis (see ref. 2 for the best of them) are not wholly satisfactory in that they plead for a general process called stabilizing selection, coupled to an

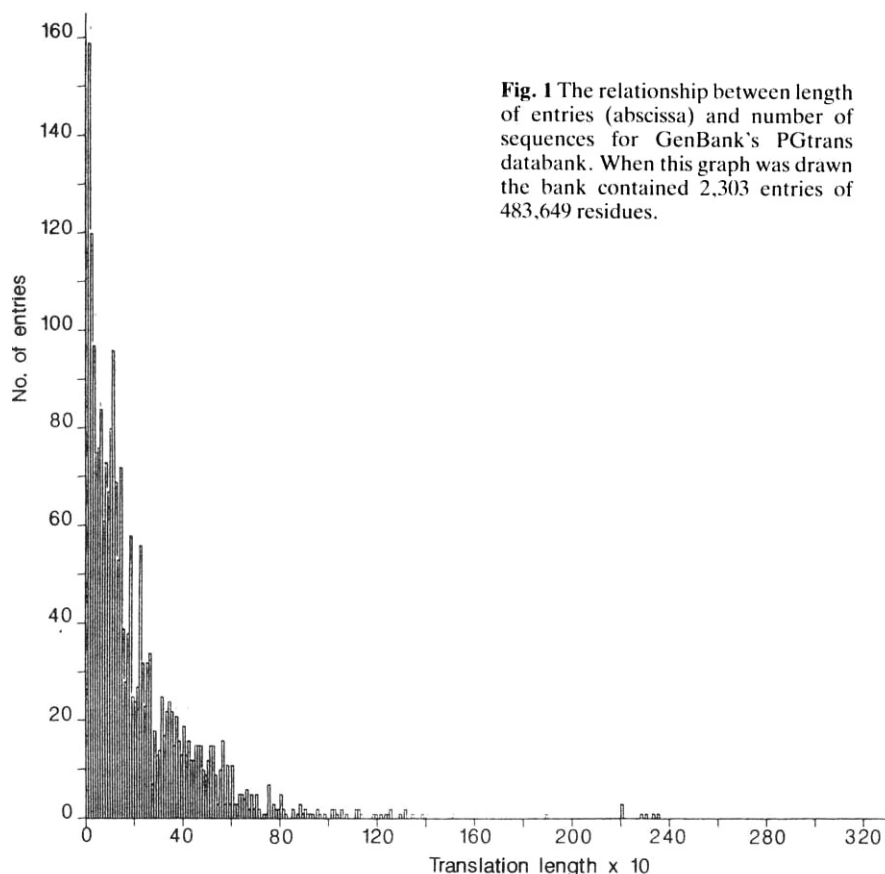


Fig. 1 The relationship between length of entries (abscissa) and number of sequences for GenBank's PGtrans databank. When this graph was drawn the bank contained 2,303 entries of 483,649 residues.

assumed inertia of developmental systems, applicable to all examples of stasis irrespective of species or conditions. This says that no evolutionarily significant shift in the average phenotype of a population took place because the ecological and developmental conditions were such that mutationally deviant individuals were continually selected against. This explanation, whilst theoretically self-evident, presupposes a uniformity of environment over vast distances of time and space that surely must be unacceptable to ecologists familiar with the ever-changing web of relationships that go into the making of ecological niches. It is difficult to accept that the heterogeneity of niches, once set up, is so stable and no longer subject to further subdivision that the usual ecological pressures, forcing variant individuals to selectively adapt to progressively specialized niches, no longer exist. Furthermore, developmental inertia is becoming the panacea for all our unsolved problems. When we don't want any appreciative directional selection to be taking place we appeal to the severity of genetic and epigenetic constraints implicated in the development of a single-celled zygote to a multicellular adult. When we do need to explain, nevertheless, that evolution is seen to be taking place sometimes, such constraints miraculously disappear.

There are other potential explanations, Darwinian and non-Darwinian, for stasis and its occasional interruption, which have not been fully explored, as yet^{1,4}. However, notwithstanding all such attempts, our general ignorance of developmental genetics and our even greater ignorance of the ecological flux and microheterogeneity of past environments, simply make it premature to wage polemical wars and to demote contemporary observations and their interpretation as "a technical parochial affair if ever there was one". The writings of Darwin, and indeed those that contributed to the formulation of the neo-Darwinian synthesis in the 1930s, are not Old Testament tracts to be poured over by armchair exegesists. They cannot supply, without resorting to too many *ad hoc* assumptions, all the answers to the multiple causes of the ebb and flow of evolution. This is relevant not only to the tempo of change observed in the geological record, but also to the evolutionary consequences of the unexpected molecular dynamics of the genes themselves. Physics moved on from Newton, and biology might need to move on from Darwin, if we are to explain, satisfactorily, all that we observe.

Dawkins is defending a tradition which hardly needs defending (for contemporary theories whilst non-Darwinian are not anti-Darwinian, despite the journalistic, and occasional professional, recourse to hype), when at the same time he is getting a little of his own back on the justifiable attacks on his own interesting little misjudgement of the "gene as the unit of

selection" (selfish gene). Ironically, this technical blunder, (despite Dawkins' protestation that it "has enriched the synthetic theory"), stood Darwin on his head and sent many a true Darwinian rushing to set the old gent firmly on his feet. Dawkins' style seems to be a self-conscious attempt to emulate the rapier-like thrusts of Sir Peter Medawar in his prime, who demolished bogus scientists with unremitting glee. Unlike Sir Peter, however, we are left with the image of a man who in largely ignoring the real scientific issue under review, is shadow boxing with himself: a stance not much help for an informed evaluation of evolutionary processes.

GABRIEL A. DOVER

Department of Genetics,
University of Cambridge,
Cambridge CB2 3EH, UK

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The length of myosin subfragment-one

SIR—A recent *News and Views* comment¹ on electron microscopy² of crystals of isolated myosin heads (S1) emphasized that these results yielded a length supporting earlier rotary shadowed views of intact myosin and S1 molecules. Results obtained from X-ray scattering³ and (by implication) certain electron microscopic acto-S1 reconstructions which yielded shorter structures were rejected as incorrect. We contend that because of a confusion arising from comparing two different kinds of lengths, there is little or no evidence for either support or rejection.

Winkelmann *et al.*² report at least 160 Å for the 'length' of S1. Measurements from their projected images (corrected for depth) indicate that this is a *contour length* and that the *maximum chord* ($l_{\max}$) observable within their structure is 120–140 Å long. The lower limit arises from the contour drawn in Fig. 11a of ref. 2. The upper limit derives from the assumption that there are crystal contacts along the long direction of the molecule. While we agree that the contour in Fig. 11a probably underestimates the volume, the data do not necessarily lead to the conclusion that the missing mass is along the long direction of S1; the molecule could just as well pack in the crystal without contacts along this direction.

X-ray scattering results give an $l_{\max} = 120 \pm 10$ Å and acto-S1 reconstructions give $l_{\max} = 115$ –150 Å (see Table 1 of ref. 4) which are consistent with the crystal values. (The largest value in this range arises from scallop S1 with regulatory light chain.) Although these results, as well as the crystal results, could underestimate the true length of the myosin head (for different reasons), the current S1 crystal results do not require that the $l_{\max}$ (190 Å) of the entire myosin head^{5,6} or S1⁷ esti-

mated from rotary shadowing projections be correct. It is nevertheless encouraging that there are features of S1 on which nearly all results concur: a curved shape with most of the mass located near one end.

ROBERT MENDELSON

Department of Biochemistry
and Biophysics,
University of California,
San Francisco, California 94143, USA

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Control of the cell cycle in yeast

SIR—In a recent *News and Views* article on yeast cell biology (*Nature* **316**, 678), I. Herskowitz states that mutations in yeast that result in G₁ arrest with reduced protein synthesis are relatively unlikely to reflect specific cell-cycle controls, in contrast with mutations such as *cdc28*, which induce G₁ arrest without reduced protein synthesis. There are several reasons for viewing this statement with caution.

First, successfully meeting the criteria of continued protein synthesis for G₁ arrest mutants does not exclude the possibility that the mutation may effect some continuous aspect of metabolism unrelated to growth control *per se*. For example, a mutation in the gene encoding ribonucleotide reductase could in principle mimic the effect of exogenously applied hydroxyurea, which can lead to G₁ arrest with continued protein synthesis.

Second, there is a wide body of evidence which suggests that attainment of a critical amount of protein is necessary for traversal of the G₁ period, and it seems relatively safe to assume that cell cycle control points operate both before and after such an event. There is no *a priori* reason that physiologically important cell cycle controls are more likely to operate after this event; in mammalian cells, G₁ arrest induced by depletion of polypeptide growth factors is usually accompanied by a marked fall in protein synthesis.

Finally, two of the mutations in *S. cerevisiae* that lead to G₁ arrest with reduction of protein synthesis, *cdc25* and *cdc35*, are now thought to be specific signal transduction components of the cAMP pathway. These gene products might exert their growth-regulatory effect by influencing protein synthesis, or a feedback mechanism might slow protein synthesis in response to inactivation of these gene products. In any case, any comprehensive view of cell cycle control should not exclude such regulatory proteins from consideration.

SCOTT POWERS

Cold Spring Harbor Laboratory,
New York 11724, USA

The politics of pesticides

Eric Ashby

Pesticides and Nature Conservation: The British Experience 1950 – 1975.

By John Sheail.

Oxford University Press: 1985. Pp.276. £19.50, \$29.95.

THE various messages conveyed by this book are important. But just as important are the seven pages at the end which carry the sources from which it was written — nearly 300 references, practically all of them from the files of the Nature Conservancy, a body set up under royal charter as the fourth of Britain's research councils in 1949 (added to the Department of Scientific and Industrial Research, the Medical Research Council and the Agricultural Research Council).

What is unusual about that? It is that the files were opened to John Sheail, even though most of them were less than 30 years old (in Britain, a ban prevents access to the papers of official government organizations for that period). How this miracle of diplomacy was achieved in a country that has no Freedom of Information Act, and which is paranoid about the disclosure of information less than 30 years old, the author does not explain. But the benefits of this relaxation are clear. The recent history of pesticide control is now public knowledge; in part, at any rate, for of course the files of the relevant Ministries may remain closed until the year 2015 (for matters being discussed today) and the files of the industries that produced pesticides are never likely to see the light.

The value of having this sort of information made public is that people concerned to influence environmental policy can find guidance on how to do it and (from many episodes described in this book) how *not* to do it. Nothing but good can come from disclosure of the complexities and legitimate conflicts which have to be resolved before decisions about the environment can be made (though only a reckless optimist would hope that the Department of the Environment might be shamed into releasing some of its papers to historians). In this sense the book is a landmark and those who had the courage to authorize the files to be opened are to be congratulated.

Skimmed over, the book itself is a sober, detailed account of the use and control of insecticides and herbicides in Britain, as seen by the Nature Conservancy and with emphasis upon the part played by the Conservancy in solving the scientific problems and interpreting the solutions for politicians. In rough outline it is a familiar story, for "every schoolboy knows" the gist of the message in Rachael Carson's *Silent Spring*. But to put the book aside with the conclusion that it is no more than a parochial account of Britain's

experience in pesticide control would be a misjudgement. True, it is a case-study of environmental decisions for one narrow issue in one small country. But it is out of such case-studies that general principles emerge, and the intricate details demand close reading: the distinction between correlation and causation in field observations; the subordinate part scientific evidence plays in some political decisions; the resolution of conflicting self-interests when there is "right" on both sides.

The story Sheail has to tell is best illustrated by the several themes which characterize his case-study. One of them is the difficulty of linking cause and effect in ecology. Forty years ago the *Atlantic Monthly* published an article by V.B. Wigglesworth, a British entomologist, about the possible side effects of DDT; it might, thought Wigglesworth, kill the predators of the very pests we want to destroy, when the predators might, in the long term, be more effective agents of control. It was only concern that DDT might be a hazard to human health — not to other organisms — that prompted official enquiry into its effects, and not until the 1950s that Wigglesworth's warning took root. By that time, however, it was clear that millions of pounds worth of crops were being saved in England and Wales by the application of insecticides, and large profits were being made by the manufacturers of sprays and equipment. There would have to be compelling reasons for any government to restrict this practice.

It was left to the Conservancy and to voluntary bodies such as the Royal Society for the Protection of Birds to find those reasons. There was an increase in the numbers of dead birds and mammals found after spraying; but it was easy to explain this away by saying that the public-

ity given to bird-deaths stimulated volunteers to look for dead birds, and the cause of the increase in numbers of dead birds was simply the increase in the numbers of people looking for them. Challenged to produce credible evidence that the birds and mammals had been killed by pesticides the Conservancy was obliged to set up its own research unit, with pitifully inadequate funds, to tackle problems which were insoluble without more refined techniques in analytical chemistry than were available at the time. It was not until the early 1960s that the Conservancy's research unit was able to publish analyses for pesticide residues in the bodies of dead birds, showing that these residues were higher among raptorial and fish-feeding birds, whose diet was contaminated by lower concentrations of the residues; it confirmed the hypothesis of concentration of residues in the food-cycle. The peregrine falcon was the protagonist in this drama. Its principal prey was live birds; these (with the irony of Greek drama) were the falcon's undoing. By 1962 its pre-war population was halved. But by then the government had accepted the scientific evidence. Persistent organochlorine pesticides were restricted, and the peregrine falcon population is well on the way to recovery.

A second theme of the book is the contribution voluntary bodies have made to the control of pesticides. It was a joint committee of the British Trust for Ornithology and the Royal Society for the Protection of Birds that hired chemists to do post-mortems on birds thought to be killed by organochlorines or mercury on seed dressings. The committee was able to show a clear correlation between sowing programmes and mortality in many parts of England. The record of voluntary conservation bodies in Britain is in every way as impressive as the efforts of the Sierra Club and the Audubon Society in America, but with this difference: in Britain the voluntary bodies have worked by bringing informal pressure to bear on the government and the public, while in the United States the voluntary bodies have frequently had to resort to law. In the United States the battle against organochlorine

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Crop spraying with pesticide — the story is not over yet.

pesticides, for example, was won through the Environmental Defense Fund.

A third theme also is concerned with voluntary effort, and it is peculiarly British. The control of pesticides comes under a so-called Pesticides Safety Precautions Scheme which has persuaded the manufacturers voluntarily to abandon some pesticides altogether. From time to time governments are pressed to impose mandatory control (and there is still a need for it in some non-agricultural uses of pesticides), but every time the matter is looked into, the advice given (even by the Royal Commission on Environmental Pollution in its Fourth Report in 1974) is that there is no case for replacing the voluntary system.

Finally, Sheail's book has some vivid illustrations of the familiar pattern of events in environmental politics, a pattern which anyone who wants to take part in such activities has to understand. There is a prologue of silent scientific work to which politicians, even if they know about it, are indifferent. There follows a sensa-

tional episode, such as the *Torrey Canyon* disaster, or an emotive popularization of some hazard, such as was so successfully done in *Silent Spring*. (It was the chairman of a Congressional subcommittee on agriculture who described the public opinion left by *Silent Spring* as "the worst residue problem we have to face today".) The cautious scientists, striving to solve the problems, are often bitterly criticized during this emotive phase. Then come pressures for legislation or, in the United States, lawsuits; and finally there has to be a compromise between those who want to exploit the environment for profit (either by growing crops or making pesticides) and those who want to protect it.

Above all, John Sheail's book is a record of missionary work by scientists among civil servants and politicians. There is no climax, but nor should there be: the story is not over yet. □

Lord Ashby is a Fellow of Clare College, Cambridge CB2 1TL, UK. He was Chairman of the Royal Commission on Environmental Pollution from 1970 to 1973.

Viewed from above

Melbourne G. Briscoe

Introduction to Satellite Oceanography. By G.A. Maul. *Martinus Nijhoff*: 1985. Pp.606. Dfl.220, \$80.

Satellite Oceanography: An Introduction for Oceanographers and Remote-sensing Scientists. By I.S. Robinson. *Ellis Horwood*: 1985. Pp.455. £42.50, \$63.75.

Methods of Satellite Oceanography. By Robert H. Stewart. *University of California Press*: 1985. Pp.368. \$38.50, £32.75.

IT WAS not so long ago that the proper background for a physical oceanographer was deemed to lie more in one's heritage and sea legs than in any particular formal education. Experience and practical apprenticeship were far more important than classes and books. In recent years it has been seen to be necessary to complement that approach of thoughtful explanation and observation with a good dose of common sense about electronic and mechanical instruments, and a solid grounding in mathematics and physics. Such mandatory education now includes the need for more than a passing knowledge of numerical methods, thermodynamics, synoptic and boundary-layer meteorology, and, depending on the chosen specialization, chemistry, geology and biology.

In this rapid evolution and expansion of formal training, however, it has been easy to forget that oceanography — even today's physical oceanography of potential vorticity balances and dispersion relations — is still mainly an observational science. Our ideas about how the ocean works are driven by what we see of its workings.

These three books bring this issue back into the limelight. They all attempt to display and explain the enormous potential of remote sensing of the oceans, principally from satellites, and to provide the information one might need to use and to understand this newly available tool. Maul aims his book at "a wide range of oceanographers, managers, and engineers" (is this a hierarchy, I wonder, and, if so, which way does it go?); Stewart says his is for "scientists with varied backgrounds"; and Robinson addresses his more eclectic book to an equally broad audience: "practising oceanographers . . . sensor technologists and remote-sensing specialists . . . and students of both oceanography and remote sensing".

The content and success of each of the books reflect the authors' backgrounds. Maul and Robinson both specialize in the infrared and optical properties (such as colour) of the sea surface, while Stewart concentrates on radio and radar scattering from the surface. Robinson's book provides some useful material on orbits and data handling, much of which is probably ephemeral, whereas Stewart has tried to include fundamental and perhaps more durable material about the basic physics of the sensors and the associated electromagnetic emission and scattering processes. Stewart's book is the most pleasing typographically, and includes a number of impressive colour plates which illustrate certain applications. Maul contains considerable detail and comes the closest to being self-contained on a number of topics. Robinson gives a lot of help to the person actually working with the data (not one of his intended audience, by the way), but a large number of typographical errors lessens one's confidence in some of his material.

In books such as these, it is unlikely that the reader will start at the beginning and read to the end; a comprehensive and detailed index is therefore essential. As a test, I tried to look up the established accuracy of sea surface temperatures as measured from a satellite, which is one of the oldest and most useful products of remote sensing. Only Robinson allows direct access to the topic via the index (but under the heading "SST"). Stewart does not even have an entry under "Temperature", although his discussion of the topic is arguably the best of the three. (Apparently, if one works very hard, it is possible to get about 1K accuracy.)

The authors are all successful in their goal of making remote sensing more accessible to the oceanographer, perhaps more so as textbooks — they are all based on course notes — than as reference books. Robinson, however, mostly fails in his attempt to provide an oceanographic background for the remote sensor; an entire book would be necessary for this task, and would be very difficult to write, but such a volume is clearly needed to stem the trend of space engineers publishing second-rate oceanography in non-oceanographic journals and in non-refereed conference proceedings.

But I wonder if these books are really necessary. Even if they meet their stated goals, was the goal worth addressing? Traditionally, a new tool becomes known to the science community by its being used and by publication of the results in science journals. These authors argue, however, that here the tool (sensors on satellites) is so different, so complicated and so inaccessible in terms of its construction and its data handling, that the issue must be forced, rather than just being allowed to happen. I suppose they are right, but you cannot force good science and it is good science based on remote sensing that will ultimately sell the new tool.

There is considerable promise in satellite oceanography as a broad but not detailed observational tool; this is precisely the missing dimension in traditional oceanographic measurements, so with perseverance by all and tolerance by the traditionalists this new view may yet pay off. These books are a step in the right direction. □

Melbourne G. Briscoe is an Associate Scientist in the Physical Oceanography Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA.

New in paperback

● *The Nature of Selection: Evolutionary Theory in Philosophical Focus* by Elliott Sober. Publisher is MIT Press, price \$9.95, £9.95. For review see *Nature* 314, 680 (1985).

● *Climate and History: Studies in Past Climates and Their Impact on Man* edited by T. M. L. Wigley, M. J. Ingram and G. Farmer. Publisher is Cambridge University Press, price £15, \$24.95. For review see *Nature* 298, 499 (1982).

Picking the winners by number

David E.H. Jones

The Awards of Science and Other Essays. By Eugene Garfield. *ISI Press, Philadelphia/STM Distribution, Enterprise House, Ashford Road, Ashford, Middlesex TW15 1XB, UK: 1985. Pp.572. \$30, £26.*

CURRENT CONTENTS, which every week reproduces the title pages of a selection of learned journals, must be the tersest and most functional magazine in existence. Almost its only concession to literature (as opposed to the literature) is the section "Current Comments", in which the editor, Eugene Garfield, looks up from his photocopier and discourses on whatever topic has taken his fancy that week. He has been publishing collections of his "Comments" since 1973, under the general title *Essays of an Information Scientist*; this book is the seventh in the series.

Of the 61 essays here reprinted, nine deal with recent research on biomedical topics such as cystitis and gerontology, and ten are tributes to notable information scientists or recent Nobel Prize winners. There is also a description of an artwork (with colour plate), a discussion of science books for children, and two articles by other authors which Garfield comments upon. But most of the essays deal with aspects of information science: in particular the services provided by the Institute for Scientific Information, founded by Garfield in 1960, and analyses of its compilations of scientific citations which are published as *The Science Citation Index*.

Most researchers, I imagine, use the *Index* regularly, if only to look themselves up and find out how they are doing: how often their most recent papers have been cited, and by whom. Computer technology not only enables the enormous lists of citations to be compiled and indexed, but also allows Garfield to indulge in his favourite hobby of scanning the lists for highly cited papers. Thus the book includes accounts of the hundred most cited papers ever; a hundred "citation-classics" from *The Lancet*, and another hundred from *The New England Journal of Medicine*; several compilations of the articles most cited between 1961 and 1982; and several essays on the citation-patterns of different scientific disciplines.

Each such essay contains a wealth of statistical material on papers and their citation-rates, the ranking of journals by citation-frequency in the rest of the literature, the number of citings of "classic" (highly-cited) papers as a function of time and so on. Garfield uses this material as a springboard for discussing developments in the field involved, and its current "hot

topics", usually buttressed by explanations from the principal authors concerned. Intriguing "maps" of the scientific frontiers are sometimes included, based on the flow of citations between the various authorities.

All this is splendid stuff, and gives a lot of information about the most active (or at least the most verbal) areas of science. However, Garfield's preoccupation with picking winners tends to give a one-sided impression of what science is all about. Thus the title essay "The Awards of Science" deals with just that; the scientific prizes and awards available to scientists, and how many of the recent prize-winners have been spotted among ISI's "most-cited authors". The object of the essay is to dispel the impression that the Nobel Prize is the only possible goal for a scientist — there are many other prestigious

prizes to compete for. "To set a practical limit for the essay, we have only included those awards that bestow an honorarium of at least \$15,000."

This image of science as a sort of competitive hit-parade is probably an occupational bias of the computerized citation-analyst. Fortunately Garfield's interest in science transcends this limited view. His concise accounts of research in many fields, and of the achievements of many scientists, are well worth browsing through for their erudition and informativeness — and, as one would expect, they are impeccably, not to say obsessively, documented and referenced. □

David E.H. Jones is a guest staff member in the Department of Physical Chemistry, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, and a science consultant to industry and the media.

The moving spirit

Simon D.M. White

Gravitational Physics of Stellar and Galactic Systems. By William C. Saslaw. *Cambridge University Press: 1985. Pp.491. £50, \$90.*

As William C. Saslaw points out in his new book, gravitation is the spirit which underlies almost all astronomical phenomena. Rightly, then, the study of gravitational dynamics has had a major part in astronomy since the time of Newton, and today it is the foundation on which we build our understanding of the structure, formation and evolution of planetary systems, of star clusters, of galaxies, and of the large-scale distribution of galaxies. Recent research has been greatly stimulated by the advent of computers that are able to integrate the equations of motion directly for systems containing thousands of particles. As a result gravitational dynamics has become, in some part at least, an experimental science.

In view of its importance in modern astronomy, it is surprising that there is no recent all-embracing account of the subject. *Gravitational Physics of Stellar and Galactic Systems* thus addresses itself to a well-recognized gap in the literature. As the title suggests, Saslaw is here more interested in elucidating physical processes than in modelling astronomical systems. His book is written in an easy-going conversational style, and is laid out as a survey of the phenomena which govern the gravitational evolution of many-body systems.

The whole field is divided into four general areas: homogeneous systems, infinite inhomogeneous systems, finite spherical systems and finite flattened systems. The first part discusses descriptive formalisms such as the Liouville, Boltzmann, Fokker-Planck and BBGKY hierarchy

equations, together with fundamental processes such as collisional relaxation, dynamical friction, collective scattering and Landau damping. The second part concentrates on clustering in an infinite universe, with particular emphasis on thermodynamic considerations, while the third deals with the structure of spherical systems and their evolution under the influence of collisional relaxation and binary formation. The last part discusses some aspects of disk galaxy dynamics and a few other assorted topics. The sophistication of treatment and completeness of coverage decrease steadily from one part to the next, even though the relevance of the subject matter to contemporary research increases. The book restricts itself to processes involving purely Newtonian gravity.

On the dust jacket, the book is described as suitable as a text for graduate students. However the depth and completeness of coverage are too patchy and too idiosyncratic to serve students well. Thus there are long and involved calculations of collective scattering and of the growth of clustering from an initially unclustered state; these are difficult to follow and lead neither to astronomically useful results nor to a significant sharpening of physical intuition. On the other hand, the treatment of the structure and instabilities of ellipsoidal and disk-like systems is at best sketchy.

Overall, the selection of material reflects the author's own research interests, and the distribution of emphasis differs somewhat from that typical of modern research in the field. The book's length is a consequence of its enjoyable but discursive style, rather than a reflection of the completeness of its contents. Unfortunately, we shall have to go on waiting for a comprehensive discussion of modern gravitational dynamics. □

Simon D.M. White is an Associate Professor at the Steward Observatory, University of Arizona, Tucson, Arizona 85721, USA.

Author and augur in theoretical physics

John Stachel

Wolfgang Pauli: Scientific Correspondence with Bohr, Einstein, Heisenberg a.o. Vol. II: 1930–1939. Edited by Karl von Meyenn. Springer-Verlag: 1985. Pp. 783. DM 298, \$112.

LIKE its predecessor (Vol I: 1919–1929), this book is indispensable reading for anyone seriously interested in the history of theoretical physics in this century. In addition to his own very significant theoretical contributions, Wolfgang Pauli was one of the “augurs”, as Einstein called them, whose judgement of new ideas is so important for their acceptance or rejection by the physics community. Indeed, Pauli came more and more to assume the role of the “conscience of physics”, now denied Einstein because of his heretical views on quantum mechanics (Pauli’s nickname — “Zweistein” — bears witness to this transfer of *mana*). “No form of approval could be more precious to physicists, not excluding Bohr, than Pauli’s benevolent nodding” Leon Rosenfeld recalled. Not that Pauli’s judgements were always definitive. In 1931, for example, he wrote to Peierls: “About semiconductors, one shouldn’t work on them, it’s a filthy mess [*Schweinererei*], who knows if semiconductors even exist” (p.94). More often, however, his views prevailed, even when he opposed other “augurs”. His prompt rejection of Bohr’s attempt to explain the continuous energy spectrum of electrons in β -decay (“The idea of a renunciation of energy conservation in the β -spectrum is and remains in my opinion a cheap and quite clumsy philosophy!”), p.4) helped lead Pauli to his greatest triumph: the prediction of the neutrino.

While the first volume of correspondence centred around discussion of the foundations of non-relativistic quantum mechanics, this collection focuses on relativistic quantum mechanics, quantum field theory and what we now call elementary particle physics. Almost half of the 362 letters are exchanges with Heisenberg. Other major correspondents (over ten letters) include Bohr, Dirac, Ehrenfest, Kemmer, Klein, Peierls and Weisskopf. (There is also a 15-item supplement to Vol. I.) Many of the letters deal with technical developments in the evolving quantum theory of fields and should be read in conjunction with the relevant published papers of Pauli and others. Editorial notes to the letters provide references to the papers; the facsimile edition of Pauli’s *Collected Scientific Papers*, and a similar edition of Heisenberg’s *Gesammelte Werke* now being issued, facilitate such comparisons, though one hopes that in future coordin-

ated editions of writings and correspondence will provide cross-references.

The extensive discussion of Dirac’s hole theory makes the absence of an edition of Dirac’s papers keenly felt. Pauli’s unhappiness with the hole theory seems to have been a driving force behind his attempts to develop a quantum field theory in which pair creation would emerge naturally. When he and Weisskopf were able to provide such a theory for scalar bosons, Pauli referred to it as their “anti-Dirac paper” (p.333). Another area in which Pauli was stimulated by dissatisfaction with Dirac’s work was the theory of higher-spin particles. Working with Fierz, Pauli first believed he had shown that such a theory was impossible for particles of spin greater than one, but then realized how to do it.

The only major discussion of the foundations of quantum mechanics in this volume is in response to Einstein’s challenge, in the well-known Einstein–Podolsky–Rosen (EPR) paper, to the completeness of the description of physical reality offered by the theory. Pauli informed Heisenberg of the paper with a characteristic example of his *politesse*: “Einstein has once again expressed himself publicly on quantum mechanics. As you know, this is a catastrophe every time it happens” (p.402). Yet Pauli immediately grasped the importance of the issue of non-separability raised by the paper: “Quite independently of Einstein . . . in a systematic foundation of quantum mechanics, one should start more from the composition and separation of systems . . . This is indeed—as Einstein has *correctly* sensed—a very fundamental point . . .” (p.404). Pauli encouraged Heisenberg to reply to the EPR paper, which he did in a previously unpublished manuscript printed on pp. 409–418.

While Pauli functioned well as a “conscience of physics”, he never took over Einstein’s role as “conscience of humanity”. His letters are notably shallow in their references to political events of a decade that spanned the world economic crisis, the advent of fascism in Germany and the crises in the Rhineland, Spain, Austria and Czechoslovakia which led to the outbreak of the Second World War. Pauli is clearly opposed to the rise of fascism, as well as the totalitarian turn taken by the Russian Revolution; but his references to such events primarily concern attempts to mitigate the personal difficulties (for some, like Heisenberg) and tragedies (for others, like Kottler) of fellow physicists or relatives, rather than any broader political perspective. The outbreak of the war caught him by surprise: “I didn’t believe in this war up to the last moment . . .” (p.678). His response is revealing of his sense of obligation and his priorities: “I think it is *my* job to take care for the continuation of the spiritual life in a time like the present one” (p.679).

A sampling of the letters suggests that

there are a large number of misprints, including garbled formulae which make certain letters (for example No. 541) difficult to read. There are also numerous misprints in the annotations, particularly in the transcription of English texts which are sometimes unintentionally humorous (Pauli’s broken arm comes from “slipping on a swimming dock” on p. 85; it is soon moved from a “splint into a cling” on p.92).

A great deal of effort has gone into the editorial annotation, much of it to good purpose. However, some questions must be raised. Detailed information about specific references in a letter is often included in an introductory note. This re-

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Star cast — Pauli (top, centre) at the Solvay conference of 1927 with Heisenberg on his left. Bohr and Einstein were also present.

quires frequent footnotes to letters directing the reader back to headnotes. One wonders whether it would not have been better to avoid such annoying interruptions by putting the necessary information into the footnotes in the first place. The sheer quantity of annotation shows a curious decay law. In each of the published volumes, about one-half of the pages in the early part is devoted to introductions and footnotes (since these are printed in smaller type than the main texts, this means the ratio of commentary to text is well over one to one). The number of pages of commentary falls off steadily as the volumes progress, until it reaches about 15 per cent of the total near the end. Did the editor’s enthusiasm slacken, was he under pressure to finish off each volume or is there some other explanation? In any case, the total amount of annotation seems excessive, and much could be pruned without great loss to the reader. An editor is under no obligation to share the fruits of *all* his or her research with the reader. One does not really admire a museum where the descriptions furnished by the staff begin to divert attention from the objects to be studied. □

John Stachel, 745 Commonwealth Avenue, Boston, Massachusetts 02215, USA, is Professor of Physics at Boston University, and editor of the *Collected Papers of Albert Einstein*, the first volume of which appears next year.

Hubble's constant determined using very-long baseline interferometry of a supernova

N. Bartel*, A. E. E. Rogers†, I. I. Shapiro*, M. V. Gorenstein*, C. R. Gwinn*,
J. M. Marcaide‡ & K. W. Weiler§

* Harvard-Smithsonian Center for Astrophysics, Cambridge, Massachusetts 02138, USA

† NEROC Haystack Observatory, Westford, Massachusetts 01886, USA

‡ Max-Planck-Institut für Radioastronomie, Bonn, FRG

§ National Science Foundation, Washington DC 20550, USA

Determination of the angular expansion velocity of the radiosphere of the supernova SN1979c in the galaxy M100 in the Virgo cluster, combined with determination of the radial expansion velocity of the photosphere, yields an estimate of the distance to the Virgo cluster centre of $D_{\text{Virgo}} = 19^{+8}_{-6}$ Mpc and of the Hubble constant of $H_0 = 65^{+35}_{-25}$ km s⁻¹ Mpc⁻¹, where the uncertainties given are based on conservative, but reasonable estimates of all significant errors and are intended to represent 90% confidence intervals.

THE supernova SN1979c in the galaxy M100 (NGC4321) in the Virgo cluster has been emitting several millijanskies of radio radiation at 5 GHz, starting about 1 year after the explosion¹. Figure 1 displays a radio map of the galaxy; the supernova is situated at the southern edge of a spiral arm. Optical spectral analysis² shows that the radial expansion velocity reached values of the order of 12,000 km s⁻¹. At a distance of, for example, 20 Mpc, this expansion velocity is equivalent to a change in the angular diameter of the exploding star of 0.25 arc ms yr⁻¹. Such a rate of change of the angular diameter of a supernova can be monitored with the radioastronomical technique of very-long-baseline interferometry (VLBI), if the presently most sensitive and most widely spaced antennas are used, and if all of them are equipped with the broad band Mark III VLBI system³.

The determination of a supernova's angular expansion rate, coupled with the determination of its radial expansion rate, allows inference of the distance to the supernova and, with its redshift corrected for local streaming motions, inference of H_0 (ref. 4). This article presents such determinations and inferences from observations of the supernova SN1979c. Preliminary results have been given earlier⁴.

VLBI observations

We made VLBI observations of SN1979c at several epochs after the time of explosion, which we assume to be 1 April 1979, about 19 days before maximum light. The observations, and the abbreviations used below, are summarized in Table 1.

At each station a hydrogen maser frequency standard was used to govern the local-oscillator chain and the 'time tagging' of the recorded data. All observations were made with the most sensitive VLBI system available [Mark III in recording mode A (see ref. 3)]. We used a bandwidth of 56 MHz, except for observations involving station Y, whose intermediate-frequency filters limited the bandwidth to 48 MHz. The data were correlated with the Mark III VLBI processor of the Haystack Observatory, located in Westford, Massachusetts.

Each of the observations of SN1979c was straddled by observations of the calibrator sources OJ287 and/or OQ208. With the clock-epoch and clock-rate information determined from the observations of the calibrator sources, we limited the search for fringes of the supernova to delay and delay-rate ranges that corresponded to windows on the sky with widths as narrow as possible but of at least 1 arc s in extent. The window

centres corresponded to the supernova position determined previously with the VLA⁶. (The positions of many celestial sources determined with the VLA (Very Large Array) agree with those determined by VLBI to within 0.3 arc s; see ref. 5 for comparison of position determinations.) The narrow search windows allowed the reliable detection of far weaker fringes than is possible when the search must extend over many independent delay and delay-rate channels to account for uncertainties in the relative clock epochs and rates (see ref. 7).

When fringes were detected, we used the corresponding information to narrow the search windows even further. In cases where fringes were detected with two two-element interferometers of a triplet of antennas, closure observables were calculated for the third two-element interferometer. The values of these observables have a probability of ~1% of being due to noise alone. For observations of SN1979c on 1 December 1983, these procedures allowed us to quadruple the number of detections relative to the number obtainable with 'normal' processing. From observations on 9 May 1983, at 8.4 GHz, we detected the source only with the BM interferometer. For the DM interferometer, we determined a 3 σ upper limit ($\sigma \approx 1$ statistical standard deviation) for the correlation coefficient, a value that corresponds to a probability of 10% that noise somewhere in the search area of 0.3×0.6 arc s would exceed this upper limit. The other upper limits were ignored in the further analysis, as they were too high to be of use.

The correlation coefficients were corrected for any coherence losses and for the bias arising from the fact that they are always positive⁸, and were calibrated to yield correlated flux densities⁹.

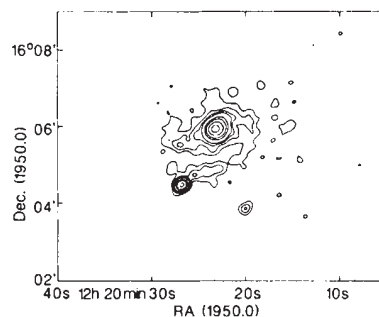


Fig. 1 Radio map of the galaxy M100 showing the supernova SN1979c [$\alpha(1950.0) = 12^{\text{h}} 20^{\text{m}} 26.71^{\text{s}}$, $\delta(1950.0) = 16^{\circ}04'29''.5$] situated at the southern edge of one of the galaxy's spiral arms. The contour levels are at 2.5, 5.0, 7.5, 10, 20, 30, 50, and 80% of the peak flux density per beam area of 9.75 mJy.

|| Present addresses: Siemens AG, Postfach 700074, München, FRG (J.M.M.); E. O. Hurlburt Centre for Space Research, Naval Research Laboratory, Washington DC 20375-5000, USA (K.W.W.).

Table 1 Observations of SN1979c

Date of observation	Radio frequency (GHz)	Polarization*	Antennas†	MSIA‡	Sensitivity§ of MSIA (7 σ) (mJy)	No. of scans with MSIA
8 December 1982	5.0	LCP	BGOY	BY	1.7	4
9 May 1983	2.3	RCP	BDFG(K)MO(S)	DM	1.5	2
9 May 1983	8.4	RCP	B(DK)M	DM	2.1	2
1 December 1983	5.0	LCP	BG(K)OY	BY	1.7	7
31 May 1984	1.7	LCP	BGOY	BY	1.4	5

According to Weiler *et al.*⁶, the coordinates of SN1979c are: $\alpha(1950.0) = 12^h 20^m 26.71^s$, $\delta(1950.0) = 16^\circ 04' 29.5''$.

* LCP, Left-hand circular polarization. RCP, Right-hand circular polarization.

† B, 100-m antenna in Effelsberg near Bonn, FRG; belongs to the Max-Planck-Institut für Radioastronomie. We give for each antenna a representative value of the product, S_{sys} , of system temperature and inverse sensitivity in Jy for each of the frequencies in GHz: $S_{sys}(1.7, 2.3, 5.0, 8.4) = 28, 260, 47, 150$. D, 64-m antenna in Goldstone, California, USA; belongs to NASA. $S_{sys}(2.3, 8.4) = 29, 45$. F, 25-m antenna in Ft Davis, Texas, USA; belongs to Harvard College Observatory. $S_{sys}(2.3) = 1,650$. G, 43-m antenna in Green Bank, West Virginia, USA; belongs to the National Radio Astronomy Observatory. $S_{sys}(1.7, 2.3, 5.0) = 66, 590, 190$. K, 37-m antenna in Westford, Massachusetts, USA; belongs to the Northeast Radio Observatory Corporation. $S_{sys}(2.3, 5.0, 8.4) = 5900, 650, 800$. M, 64-m antenna near Madrid, Spain; belongs to NASA. $S_{sys}(2.3, 8.4) = 36, 50$. O, 40-m antenna near Big Pine, California, USA; belongs to California Institute of Technology. $S_{sys}(1.7, 2.3, 5.0) = 235, 425, 300$. S, 25-m antenna in Onsala, Sweden; belongs to Onsala Space Observatory. $S_{sys}(2.3) = 1,000$. Y, Equivalent to a ~ 130 -m antenna ($\sim 26 \times 25$ m antennas of the VLA 'phased up') near Socorro, New Mexico, USA; belongs to the National Radio Astronomy Observatory. $S_{sys}(1.7, 5.0) = 26, 27$. All two-element interferometers with the symbol for at least one antenna in parentheses failed to yield fringes.

‡ Most sensitive two-element interferometer of array.

§ Maximum sensitivity for coherent integration of 12 min and a bandwidth of 50 MHz. Here and hereafter, 1σ denotes one statistical standard deviation.

|| One scan consists of an observing time of ~ 12 min using a bandwidth of ~ 50 MHz.

Table 2 Parameter estimates for SN1979c

Date of observation	Time since explosion* (yr)	Frequency (GHz)	$S_{tot}^{VLA}\dagger$ (mJy)	S_{tot} (mJy)	Angular diameter‡ (mas)
8 December 1982	3.691	4.99	5.5 ± 0.3	$5.30^{+0.26}_{-0.54}$	$1.05^{+0.09}_{-0.10}$
9 May 1983	4.117	2.29	—	$9.7^{+0.8}_{-0.4}$	$1.67^{+0.26}_{-0.12}$
9 May 1983	4.117	8.42	3.9 ± 0.2	$3.8^{+0.2}_{-0.2}$	> 0.91
1 December 1983	4.672	4.99	4.2 ± 0.2	$4.08^{+0.45}_{-0.38}$	$1.43^{+0.07}_{-0.07}$
31 May 1984	5.173	1.67	7.9 ± 0.6	$7.75^{+0.61}_{-0.63}$	$2.16^{+0.44}_{-0.50}$

* The assumed date of explosion is 1 April 1979.

† The total flux density of the supernova measured with the VLA alone. For the 5-GHz data, the errors were taken to be 5% of the total flux density so as to include both statistical standard deviations and possible systematic errors. For the 1.7-GHz data, the error represents the variation of the total flux densities determined from observations of the source on 31 May 1984 and 1 June. In all four cases, the 1σ noise level in apparently blank fields of the maps near the position of the supernova is more than three times smaller.

‡ Estimated angular diameter for a uniform sphere model of the brightness distribution of the supernova. For the meaning of the errors, see text.

The calibration procedure was checked for our observations of the calibrator sources, which have relatively simple brightness distributions, and was found to yield flux densities consistent with the predictions from a 'best-fit' circular gaussian brightness distribution to within $\pm 7\%$ in each case.

Angular diameter determinations

The angular diameters of the supernova at the epochs of our observations were all small compared with the smallest fringe spacing obtainable with our arrays. Hence, the radio brightness distribution of the supernova cannot be well determined from our VLBI data. Further, because of the low declination of SN1979c and the mainly east-west orientation of our VLBI arrays, the highest resolution in the north-south direction was about four-fold lower than that in the east-west direction, making any (small) deviations of the brightness distribution from circular symmetry difficult to discern.

Under these circumstances, any angular diameter of the supernova inferred from the VLBI data will be strongly model-dependent. We, therefore, considered a range of plausible possibilities, assuming that any given model, if applicable at all, would be applicable for all of our epochs of observation. Clearly, a model in which the brightness is distributed in a thin circular ring, normal to our line-of-sight, will lead to the smallest inferred angular diameter. By contrast, a model in which the brightness is highly concentrated near the centre will yield a relatively large value for the inferred angular diameter.

We take as an example an optically-thin uniform-sphere

model. From the correlated flux densities, S_{corr} , from each epoch of observation, we estimated by weighted-least-squares the total flux density, S_{tot} , and the angular diameter, θ_{us} , as well as the standard errors in these estimates implied by the statistical standard errors of S_{corr} . The values of S_{corr} were normalized by dividing by S_{tot} and plotted together with the predictions of the model in Fig. 2.

We estimated crudely the contribution of possible calibration errors to the uncertainties of the parameter estimates by changing, in turn, the sensitivity of each antenna of the array by $\pm 15\%$ while keeping the sensitivities of the other antennas fixed. We consider this choice of the value for the uncertainty of the antenna sensitivities to be conservative in view of our check of the calibration procedures for the calibrator sources, mentioned above. The largest variations in the estimated parameters due to the changes of the sensitivities of the antennas were twice as large as the corresponding statistical standard deviations. For the uncertainty of each parameter estimate, we adopted the root-sum-square (r.s.s.) of the value of the largest variation and the statistical standard deviation. The results of the analysis are given in Table 2.

To check the sensitivity of our results to the choice of antennas, we repeated the analysis with data from subsets of antennas. The estimated parameters from this analysis were reasonably consistent with the parameter values obtained from the analysis of all the data⁴.

We also tried to check for any significant deviation from circular symmetry by using elliptical gaussian models. For each

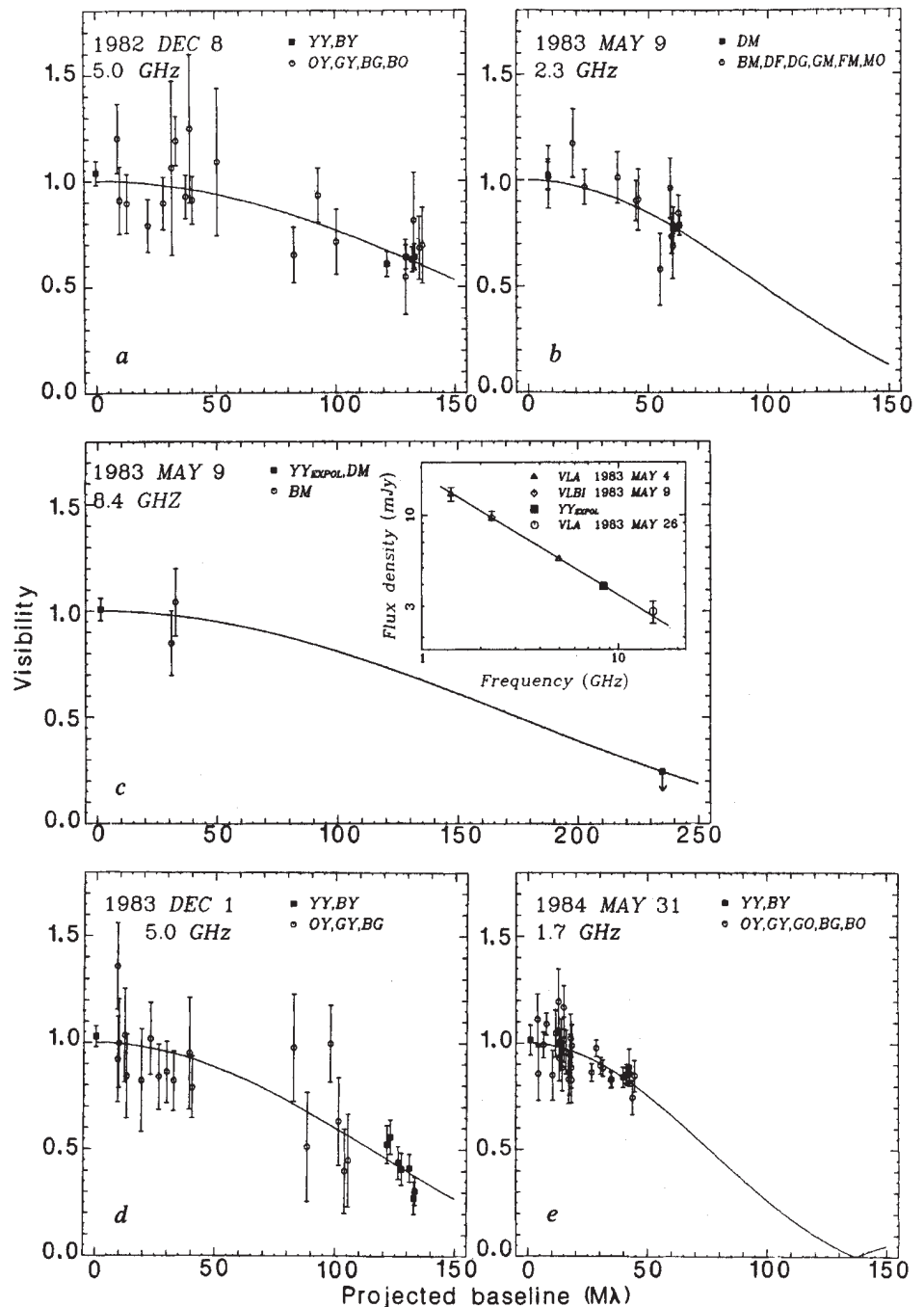


Fig. 2 *a-e*, Measured visibility amplitudes and predictions from the fit of a uniform sphere model. The filled squares show the most significant data points. Antenna pairs are listed in order of increasing baseline length. The symbol YY indicates the values for the total flux density measured with the VLA. The inset of Fig. 2c shows the weighted-least-squares fit to the spectral index, $\alpha = -0.69 \pm 0.06$ ($S_\nu \propto \nu^\alpha$), from the values of the total flux density at 1.5 and 5 GHz obtained at the VLA (ref. 1) and at 2.3 GHz obtained with our VLBI array 5 days later. The filled square shows the value for the flux density extrapolated with this power law to $\nu = 8.42$ GHz. This value is used as the zero-baseline correlated flux density (YY_{EXPOL}) in Fig. 2c. Also shown is the data point at 15 GHz obtained with the VLA (ref. 1) ~ 3 weeks later.

set of 5-GHz observations of the supernova, the ratio of the estimates of the major and minor axes was unity to within 1σ ; however, 1σ for these ratios was as large as ~ 2.5 . A higher north-south angular resolution is needed to determine, or to derive a useful upper bound on, any deviation from circular symmetry the source might have.

We also computed closure phases to search for evidence for asymmetry of the radio structure of the supernova. However, the closure phases were randomly scattered, thereby indicating no significant departure from zero. This result is also consistent with, but not conclusive evidence for, the supernova being circularly symmetric.

For a circular-gaussian model for the brightness distribution, the angular diameter at half maximum is 1.81 times smaller than θ_{us} , and at 10% of maximum about equal to θ_{us} . A thin ring, as stated above, gives the smallest diameter, 1.65 times smaller than θ_{us} . Optically thin shells give diameter determinations intermediate between those of a thin ring and a uniform sphere;

for example, for a uniform shell of thickness 5–25% of the outer radius of the shell, the diameter becomes, respectively, 1.34–1.24 times smaller than θ_{us} (see refs 10, 11).

An optically-thick shell or sphere, which is equivalent to a uniform disk, yields a diameter determination $\sim 10\%$ smaller than θ_{us} . However, since the time of our first VLBI observations, the radio spectrum of the supernova has had a spectral index, α ($S_\nu \propto \nu^\alpha$) of ~ -0.6 (ref. 12), indicating that the radio source is optically thin. Optically thick sources can therefore be ignored, but in any event do not extend the uncertainty of our diameter estimates.

An optically-thin shell of emission is favoured by Chevalier¹³, who assumes the radio radiation is synchrotron emission from the interaction of the supernova shock front with a circumstellar medium created by presupernova mass loss. The classical supernova remnant with a shell of emission is Cas A (see, for example, ref. 14). With $\sim 80\%$ of all supernova remnants (SNRs) being conventionally represented as shell-type models¹⁵, the shell

geometry seems to be the standard for the radio emission for epochs at least several hundred years after the supernova explosion.

An optically-thin uniform sphere of emission, similar to a centre-filled remnant, that is, a plerion, could be expected from one type¹⁶ of centrally driven models (refs 16, 17; see also ref. 18). The classical SNR of plerionic morphology is the Crab nebula. Only a few such examples have yet been found¹⁵. Moreover, plerionic SNRs have flatter spectra ($\alpha > -0.3$) than shell-like SNRs, which have $\alpha \sim -0.45$ (ref. 15), and thus may be less closely related to SN1979c than shell-like SNRs, if evolutionary effects on the geometry and the spectrum of a remnant do not make such comparisons irrelevant.

A ring of emission for SNRs has recently been suggested by Manchester¹⁹, who assumes interaction of a rotating pulsar beam of particles with the medium close to the boundary of the SN ejecta. We include such a geometry in estimating the uncertainty of our diameter determinations of SN1979c.

Considering all of these possibilities, we conclude that the diameters inferred from the uniform sphere and the thin ring probably straddle the actual diameter, which is likely to be that of an optically-thin shell.

Time dependence

The values for the angular diameters, θ , of the supernova for both the uniform sphere and the ring models are plotted in Fig. 3 as a function of time. The results from the 5-GHz data indicate that, with high probability, the supernova is expanding. These results, combined with the zero diameter at the time of explosion, allow the deceleration (or acceleration) to be estimated from an assumed time dependence of the angular diameter: $\theta \propto t^m$ (see refs 20, 21). Taking the standard errors for the angular diameter determinations to be independent yields the result $m = 1.3 \pm 0.5$.

Deceleration of the expansion of the supernova would be expected for the shock-front model, and evidence for it has been found in shell-type SNRs (see ref. 22). In contrast, acceleration of the expansion would be expected for the centrally-driven model and some evidence for weak acceleration has been found in the Crab nebula²³. However, neither the accuracy nor the number of our measurements is yet sufficient to discriminate between these models on the basis of our estimate of the parameter m . More observations at later epochs are required to determine with useful confidence any deviation of m from unity.

Frequency dependence

The value we obtained for the angular diameter at 2.3 GHz is significantly larger than the value for the diameter at 5.0 GHz when compared at the same epoch (we assume $m = 1$). The value at 1.7 GHz, because of its relatively large uncertainty, and the lower bound at 8.4 GHz, are both consistent with the diameter's not being a function of frequency.

Is this frequency dependence inherent to the supernova or could the angular diameter be broadened noticeably by scattering in the propagation medium? From a survey of pulsar interstellar scintillation, Cordes *et al.*²⁴ predict that the scattering angle, θ_{scat} (FWHM of a gaussian), of a source at the supernova's galactic latitude, $|b^{\text{II}}| = 77^\circ$, is between 170 and 340 arc μ s for 1.7 GHz and scales as $\theta_{\text{scat}} \propto \nu^{-2.2}$. To calculate the effect of θ_{scat} on the estimate of θ_{us} , we multiply θ_{scat} by 1.81 to convert its value to that of the 'equivalent' uniform sphere. Thus, we could, at $\nu \geq 5$ GHz, essentially exclude any broadening of the angular diameter and at $\nu \sim 2.3$ and 1.7 GHz expect broadening of no more than 2 and 4%, respectively. However, if we use weighted least squares to fit to the observed data a function of the form, $\theta_{\text{us}}^2(\nu, t) = 1.81^2 \theta_{\text{scat}}^2(\nu) + \theta_{\text{us}}^2(t)$, with $\theta_{\text{scat}} \propto \nu^{-2}$ and $\theta_{\text{us}} \propto t$ as plotted in Fig. 3, we obtain a good fit and a value for θ_{scat} (1.7 GHz) of 1.1 arc ms, which is about three-fold larger than the above upper bound of Cordes *et al.* In fact, VLBI determinations by Resch²⁵ at 74, 111, and 196 MHz of angular diameters of extragalactic sources situated within $\sim 20^\circ$ of SN1979c indicate $\theta_{\text{scat}} \propto \nu^{-n}$ with $n < 2$ and values for θ_{scat} much larger than

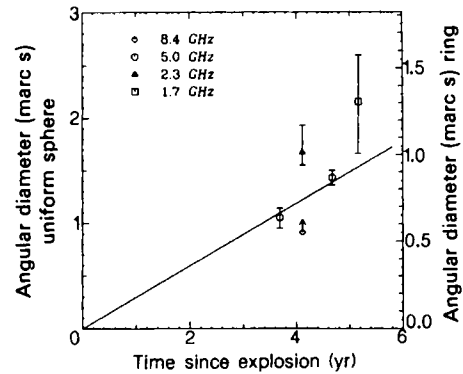


Fig. 3 The angular diameter determinations assuming two (extreme) models of the brightness distribution. The solid straight line represents a least-squares fit to the assumed zero-point origin and the two 5-GHz values. The 1.7 and 2.3-GHz values are probably subject to broadening by scattering in the propagation medium (see text).

1 arc ms when extrapolated to 1.7 GHz. Also, as Cordes *et al.* noted, some values of Resch exceeded by up to a factor of 1.6 their upper bounds even when a scattering law of $n = 2.2$ was assumed. We thus conclude that our estimate of θ_{scat} is within the range of others' estimates and that our diameter determinations at 2.3 and 1.7 GHz could have been significantly broadened by scattering in the propagation medium, but that our determinations at 5.0 and our lower bound at 8.4 GHz could not. If the angular diameter at the lower frequencies is indeed broadened by scattering, the difference between the diameter determinations at different frequencies should decrease as the source expands; future observations at more than one (low) frequency could help to resolve this issue.

Distance to M100 and Hubble's constant

To estimate the distance to SN1979c, we combine our radio determination of the supernova's angular expansion velocity with optical determinations of the radial expansion velocity. We first list the main assumptions used in making this estimate: (1) the widths of optical spectral lines reflect expansion of the supernova's photosphere; (2) the expansions of the photosphere and the radiosphere are each isotropic within a factor $\mu = 1.0 \pm 0.15$; (3) the expansion velocity of the radiosphere equals that of the outer boundary of the supernova's shock front and is, for at least 1 yr after the explosion, a factor of $\eta = 1.2 \pm 0.2$ greater than the expansion velocity of the photosphere; and (4) the angular diameter θ of the radiosphere can be described by $\theta \propto t^m$, with t the time since explosion and m constrained by $m \leq 1$ (no acceleration allowed).

The first assumption is based on the finding that in the early stages of supernova evolution the time dependences of the inferred photospheric temperature and the received flux are both consistent with an expanding black body (see, for example, ref. 26). However, to some extent, the broadening of the lines may also be caused by other processes, for example, turbulence in the emission region.

The second assumption is supported by observations that most young and medium aged SNRs are approximately circularly shaped (ref. 27 and refs therein) and by the likelihood that the degree of circular symmetry for supernovae is even higher. In the future, for the photosphere, optical spectropolarimetry might reveal any anisotropic expansion at least in the early stages of a supernova's evolution^{28,29}. For the radiosphere, any deviations from non-isotropic expansion in the plane of the sky could, in principle, be determined accurately through VLBI by mapping the brightness distribution of the supernova, but our present data cannot yield useful determinations. Our assumed uncertainty for μ is consistent with observations of most SNRs and with inferences for supernovae.

The third assumption is based on the fact that charged particles are accelerated in shock front regions and thus are likely

to radiate radio waves. In the shock front model, the radiosphere is a shell of emission with its outer radius being equal to the outer radius of the supernova's shock front and its inner radius equal to the radius of the ($H\alpha$) photosphere³⁰. In centrally driven models, the radio radiation emanates from the shock front¹⁷ or predominantly from inside the shock front shell¹⁶. For the latter case, the photospheric material is assumed to have developed a filamentary structure very soon after the explosion as otherwise the opacity of the material would prevent penetration of radio radiation³¹. We do not discuss here the likelihood of such a process; rather we assume that any emission region inside the shock front shell is not concentrated near the center but is described by a uniform sphere with its expansion velocity linked to that of the photosphere such that $\eta \geq 1$.

What is the expansion velocity of the photosphere? Optical and ultraviolet spectra of SN1979c were obtained at several epochs^{2,32-35}. The dominant profile in the optical spectrum is the $H\alpha$ emission line. The blue extension of this line indicates expansion velocities of (ref. 2) $\sim 10,500 \text{ km s}^{-1}$ on 28 May, 26 June and 19 November 1979, and (ref. 35) $\sim 9,000 \text{ km s}^{-1}$ on 10 April 1980. The minima of the absorption troughs of the P Cygni profiles of the ultraviolet and optical lines other than $H\alpha$ indicate expansion velocities between $\sim 8,800$ and $10,000 \text{ km s}^{-1}$ in the period between 22 April and 1 July 1979 (refs 2, 33). According to Kirshner³⁵, all these values define the expansion velocity of the photosphere; the maximum expansion velocity of supernova ejecta is larger. The blue edge of the absorption trough in the $\text{He I } \lambda 5,876/\text{Na I D}$ profile, in spectra taken between 22 April and 26 June 1979, reveals a velocity of $\sim 12,000 \text{ km s}^{-1}$ (ref. 2); this value probably defines the expansion velocity of the outer boundary of the supernova's shock front. Considering these various results, we adopt a value of $v_{\text{ph}} = (9 \pm 1) \times 10^3 \text{ km s}^{-1}$ for the expansion velocity of the photosphere for an epoch, say, 1 yr following the explosion, and a value of $\eta v_{\text{ph}} = (11 \pm 2) \times 10^3 \text{ km s}^{-1}$ for the expansion velocity of the radiosphere at this epoch, consistent with the maximum expansion velocity of the supernova ejecta (for $0.8 \leq m \leq 1.0$; see below).

The fourth assumption allows for the expansion velocity of the shock front to vary with time. The parameter m was estimated to be ≥ 0.89 from the change of the width of $H\alpha$ ³⁶, and ≥ 0.90 from the radio light curve³⁷, both values consistent with the inference from our VLBI observations. More VLBI observations are needed not only to determine m more accurately, but also to test our assumption that a simple power law represents adequately the expansion of the radiosphere. In fact, the range of values of m consistent with the present VLBI observations is so great as to be in large part inconsistent with assumptions (3) and (4), when the latter are coupled with the estimates, discussed above, of expansion velocities inferred from optical spectra.

Using the (assumed) relation between the velocities of expansion of the radiosphere and the photosphere at an epoch, t_{ph} , 1 yr after the supernova explosion, to determine the proportionality constant in the (assumed) relation $\theta \propto t^m$, and the relation $\theta_{\text{ph}} = 2v_{\text{ph}}/D$, where θ_{ph} is the angular velocity of expansion of the photosphere at that epoch, v_{ph} the corresponding (radial) expansion velocity, and D our distance from the supernova, we obtain by simple algebra:

$$D = \frac{2\eta v_{\text{ph}} t}{\theta m} \left[\frac{t}{t_{\text{ph}}} \right]^{m-1}$$

With $\eta v_{\text{ph}} = (11 \pm 2) \times 10^3 \text{ km s}^{-1}$ on, say, 10 April 1980 (that is, $t_{\text{ph}} = 3.2 \times 10^7 \text{ s}$), with m restricted to $m = 0.9 \pm 0.1$, and with $\theta = 1.0 \pm 0.25 \text{ arc ms}$ (see Fig. 3) at $t = 1.35 \times 10^8 \text{ s}$, the epoch midway between our two 5-GHz data points, we obtain for SN 1979c: $D_{\text{SN1979c}} = 19_{-6}^{+8} \text{ Mpc}$. If we further assume that SN 1979c is in M100 (Fig. 1) and that the distance to M100 is within 10% of the distance D_{Virgo} to the centre of the Virgo cluster, since M100 is within 6° of this centre and has a redshift consistent with this location, we get:

$$D_{\text{Virgo}} = 19_{-6}^{+8} \text{ Mpc}$$

The range of uncertainty given is intended to represent the 90% confidence interval and is based on the assumption that each of the parameters in the above expression for D contributes independently.

A value of Hubble's constant, H_0 , can be obtained by combining our distance estimate with determinations of the mean (radial) velocity, v_0 , of Virgo away from the centre of the Local Group and the correction for the infall (radial) velocity, v_{infall} , of the centre of the Local Group towards Virgo: $H_0 = (v_0 + v_{\text{infall}})/D_{\text{Virgo}}$. Unfortunately, $v_0 + v_{\text{infall}}$ is not well determined. Considering the spread in the various published values for v_{infall} , based on observations of galaxies (see refs 38-43), as well as the equivalent values estimated from the anisotropy of the microwave background⁴⁴⁻⁴⁶, and the different estimates of v_0 (see refs 47-49), we take $v_0 + v_{\text{infall}} = 1,250 \pm 150 \text{ km s}^{-1}$ and obtain

$$H_0 = 65_{-25}^{+35} \text{ km s}^{-1} \text{ Mpc}^{-1}$$

where the range of uncertainty given is dominated by that for D_{Virgo} and is also intended to represent the 90% confidence interval.

Comparing our estimates with those obtained by de Vaucouleurs⁵⁰ ($D = 11.8 \text{ Mpc}$ for M100, equivalent to $H_0 \sim 100 \text{ km s}^{-1} \text{ Mpc}^{-1}$) and by Sandage and Tammann^{51,52} ($D = 22.2 \text{ Mpc}$ for M100, equivalent to $H_0 \sim 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$), we find that our estimates are nearly midway between theirs but hardly distinguish between them.

More, and more accurate, VLBI measurements on SN1979c and other supernovae should allow us to improve the determinations of θ , m , and μ . However, we have not yet conceived of any purely observational means to relate the photosphere and the radiosphere. The uncertainty in this relation will therefore likely dominate the uncertainty in any estimate of H_0 based on the method described here, until the relation can be reliably modeled or until optical interferometry, perhaps from earth orbit, can be used to measure directly the angular size of a supernova's photosphere.

Conclusion

VLBI observations of SN1979c in the galaxy M100 yielded estimates of the angular size of the supernova. These measurements were used, together with other information, especially from the optical spectra, to estimate the supernova's distance and Hubble's constant. The range of the uncertainties of our estimates can probably be decreased with new and more sensitive VLBI observations of SN1979c in the near future. The sensitivity can be increased by recording the data on longer tapes, thus allowing longer integration times. It can also be increased by including in the array the 305-m diameter antenna at Arecibo. This antenna's low latitude would increase our north-south resolution two-fold and might enable us either to determine the brightness distribution of SN1979c or, at least, to measure, or place useful bounds on, its deviation from circular symmetry.

Observations of SN1979c at 5 GHz at a third epoch would probably be useful for a more accurate determination of the deceleration (or, possibly, acceleration) of the expansion. More observations at lower frequencies would allow more detailed investigation of whether the apparent source diameter is broadened by scattering in the propagation medium.

About one supernova per year should be detectable with our present VLBI system. Moreover, the Hubble Space Telescope should allow measurement of supernova spectra at the same epochs at which VLBI techniques could be used to measure the angular size of the supernova. Combining such measurements should enable us to determine distances to galaxies as far away as $\sim 40 \text{ Mpc}$. Observing supernovae in many different directions will allow us to study statistically the deviations of their (radial) velocities from the Hubble flow and thereby provide an indication of the accuracy of our inferred value of H_0 .

Our method is the fourth that makes use of supernovae as distance indicators (for descriptions of the other three, see refs 53-56). Each has its difficulties, but each may be useful for deriving distance estimates at least partially independent of the

others and thereby for allowing useful redundancy in the estimates of Hubble's constant.

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Magnetic confinement of a neutral gas

R. V. E. Lovelace, C. Mehanian, T. J. Tommila* & D. M. Lee

Department of Applied Physics, Department of Physics, and Laboratory of Atomic and Solid State Physics, Cornell University, Ithaca, New York 14853, USA

A three-dimensional dynamic trap for confining a collisional neutral gas is described. The trap uses the interaction of the magnetic moments of the gas atoms with a time-dependent magnetic field. Collisions lead to the evaporative cooling of the trapped atoms and to the viscous heating of the gas, particularly at high densities. This magnetic trap may allow the attainment of Bose-Einstein condensation in spin-polarized atomic hydrogen gas.

THE dynamic magnetic confinement of a neutral gas by the interaction of the magnetic moments of the gas atoms with a magnetic field including a rapidly oscillating component creates a magnetic trap which may provide a means of producing a Bose-Einstein condensation in a spin-polarized atomic hydrogen gas.

Fritz London¹ was the first to consider the phenomena of superconductivity in metals and superfluidity in liquid ⁴He in terms of quantum mechanics on a macroscopic scale. Since then, superfluidity has been found to occur in liquid ³He below 3 mK (ref. 2), and it is thought to occur in neutron stars³. The recently achieved stabilization of spin-polarized atomic hydrogen gas in strong magnetic fields (9 T) and at low temperatures (100-500 mK) presents a possible new opportunity to observe macroscopic quantum phenomena^{4,5}. Because hydrogen atoms behave as bosons, the macroscopic manifestations of Bose-Einstein condensation are expected to lead to a variety of interesting

quantum properties in this dilute, weakly interacting Bose-Einstein gas. The occurrence of Bose-Einstein condensation in an ideal Bose gas is governed by the equation

$$T_c = [h^2 / (2\pi m k_B)] (n / 2.61)^{2/3}$$

where T_c is the critical temperature, n is the particle density, m is the particle mass, h is Planck's constant, and k_B is Boltzmann's constant. Sufficiently high densities and low temperatures must be attained in order to have a Bose-Einstein condensation. Table 1a shows densities and temperatures corresponding to the onset of Bose-Einstein condensation.

Spin-polarized atomic hydrogen gas is formed by collecting atoms in the two low-energy hyperfine Zeeman states (the 'a' and 'b' states, where the electron spin is antiparallel to the magnetic field) in a strong magnetic field at low temperatures⁶. Atoms in these two states are forced towards regions of stronger magnetic field, while atoms in the two high-energy hyperfine states (the 'c' and 'd' states, where the electron spin is parallel to the magnetic field) are forced towards low-field regions. The 'a' state is a mixed state whose wavefunction includes a small

* Permanent address: Department of Physical Sciences, University of Turku, SF-20500, Turku, Finland.

Table 1 Conditions for Bose-Einstein condensation (a) and parameters for a dynamic trap (b)

a	T_c (K) = 1	0.1	0.01	0.001	0.0001
	n (cm ⁻³) $\approx 5 \times 10^{20}$	1.5×10^{19}	5×10^{17}	1.5×10^{16}	5×10^{14}
b	Temperature T (mK)	Coil radius R (cm)	a.c. current min I (kA-turns)	a.c. field b_z (O) (T)	Frequency min f_c (kHz)
	3	1	15.4	0.97	3.3
	1	1	5.1	0.32	1.9
	0.3	1	1.5	0.09	1
	3	0.5	7.7	0.97	6.6
	1	0.5	2.6	0.32	3.8
	0.3	0.5	0.75	0.09	2

admixture of the electronic spin-up state causing recombination of atoms to molecules in three-body, singlet interaction collisions (where the helium-coated surfaces of the confinement vessel may play the role of the third body). As a consequence, the sample loses its 'a' state population as time progresses, leaving only the pure 'b' state atoms whose nuclei and electrons are polarized parallel to one another (and therefore, have a triplet interaction). Because of the long longitudinal relaxation time (T_1) between the 'b' and the 'a' states, the resulting doubly polarized sample would be relatively long lived^{7,8}, and thus ideal to produce the high-density sample needed to fulfill conditions for Bose-Einstein condensation were it not for the three-body dipole recombination which becomes important at densities above 10^{18} cm⁻³ (refs 9-14). One possible solution is to obtain Bose-Einstein condensation at temperatures below 10 mK, where the required gas phase densities are less than 10^{17} cm⁻³. Unfortunately, at these temperatures surface densities are high even for liquid helium film surfaces (binding energy ~ 1 K), so that surface three-body dipole recombination destroys the gas rapidly⁹⁻¹⁴.

An alternative approach is to remove the influence of surfaces by constructing a three-dimensional magnetic trap¹⁵⁻¹⁷. Static magnetic field minimum traps are possible, but only for the 'c' and 'd' state atoms, which are low-field seekers¹⁵. One could inject a 'b' state gas and use electron spin resonance to flip the electron spins so that the resulting 'c' state atoms would be magnetically confined. However, dipole-dipole interactions are predicted⁹ to cause a rapid flipping of the electron spins and this would lead to a rather rapid loss of the sample. (Note that a variety of three-dimensional electromagnetic traps have been used for the collisionless confinement of small numbers of ions¹⁸.)

Here we investigate a three-dimensional dynamic magnetic trap, where the magnetic field includes a 'fast' time-dependent component. The dynamic trap must provide containment in the presence of frequent collisions. (Thus, one can rule out confinement in a rotating magnetic mirror field where the possible stable single-particle motion is analogous to that of the Trojan asteroids¹⁹.) A simple dynamic trap which is viable in the presence of collisions can be made by passing an alternating current through a circular wire loop located with its axis parallel to a strong uniform static magnetic field. This magnetic field oscillates between a mirror and an anti-mirror configuration and provides containment for the high-field-seeking 'a' and 'b' state atoms (as well as for the 'c' and 'd' state atoms). Here we first analyse the collisionless confinement of a single atom, then investigate the influence of collisions using the classical Boltzmann transport equation and a Monte-Carlo code for the numerical evaluation of particle orbits including collisions. (A quantum-mechanical, mean-field treatment of a collisional gas confined in a dynamic trap²⁰ confirms the classical treatment for temperatures above T_c .) Next, a treatment is given of the evaporative loss of atoms and heat from the trapped gas, and of the viscous heating of the gas. A method is described for filling a dynamic trap using the evaporative cooling which can be enhanced by gradual reduction in the depth of the magnetic well. This may allow the attainment of the conditions for Bose-

Einstein condensation. An alternative approach to Bose-Einstein condensation using a magnetic field and evaporative cooling has recently been proposed by Hess²¹. Finally, we discuss some of the anticipated problems of filling the trap and of observing the Bose-Einstein condensation in a spin-polarized atomic hydrogen gas.

Single-particle motion

The envisaged temporal variations of the magnetic field $\mathbf{B}(\mathbf{x}, t)$ are very much slower than the electron spin precession period. Thus, the potential describing the interaction of the atom with the magnetic field is

$$V(\mathbf{x}, t) = -\mu|\mathbf{B}(\mathbf{x}, t)|$$

where μ is the magnetic moment of the atom. For the case of spin-polarized atomic hydrogen, $\mu \approx \hbar e/(2m_e c) \approx 0.93 \times 10^{-20}$ erg G⁻¹ (or 0.93×10^{-23} J T⁻¹), where m_e is the electron mass. Note that the magnetic moment is parallel to $\mathbf{B}$ for the 'a' and 'b' states of atomic hydrogen so that $\mu > 0$. For the 'c' and 'd' states it is antiparallel and $\mu = -|\mu|$. In the absence of collisions, the equation of motion for an atom of mass m is

$$m \frac{d^2 \mathbf{x}}{dt^2} = -\nabla V \quad (1)$$

In a static field $\mathbf{B}(\mathbf{x})$ a spin-polarized atom with μ parallel to $\mathbf{B}$ (the 'a' or 'b' states of hydrogen) tends to move towards regions of maximum $|\mathbf{B}|$. There are, of course, no isolated maxima of $|\mathbf{B}|$ in free space (Earnshaw's theorem; see, for example, ref. 22).

The magnetic field $\mathbf{B}(\mathbf{x}, t)$ is considered to consist of a uniform, time-independent component $B_0 \hat{z}$ (with $B_0 > 0$), and the time-dependent field $\mathbf{b}(\mathbf{x}, t)$ of one or more circular wire loops with axes coinciding with the z -axis. We consider the case of a single wire loop of radius R . We assume $b^2 \ll B_0^2$ so that $|\mathbf{B}| \approx B_0 + b_z$. In the vicinity of the centre of the loop, we have

$$b_z = b_0(t) \left\{ 1 + \frac{3}{4} \left(\frac{r}{R} \right)^2 - \frac{3}{2} \left(\frac{z}{R} \right)^2 + \dots \right\}$$

where $r^2 = x^2 + y^2$, $b_0(t) = 2\pi I(t)/(cR)$ in gaussian units [or $\mu_0 I/(2R)$ in MKS units], and I is the current in the wire loop. For $b_0 < 0$ the field configuration corresponds to a mirror field, whereas for $b_0 > 0$ it is an anti-mirror configuration. The Taylor expansion simplifies the immediate discussion; the exact field of a circular loop is used subsequently. From equation (1),

$$\frac{d^2 x}{dt^2} = -\alpha(t)x, \quad \frac{d^2 y}{dt^2} = -\alpha(t)y, \quad \frac{d^2 z}{dt^2} = 2\alpha(t)z \quad (2)$$

where $\alpha(t) = (3/2) [\mu b_0(t)/(mR^2)]$. Evidently, the sign of μ is irrelevant if $b_0(t)$ is an oscillatory function with an average value of zero.

Exact solutions to equations (2) exist for the case where $b_0(t)$ or $\alpha(t)$ has a square wave dependence, that is, $\alpha(t) = \alpha_0 \text{sign}[\sin(\omega_e t)]$, where α_0 and ω_e are constants. This case is evidently the temporal analogue of an alternating-gradient or

strong focusing synchrotron²³. The motion in the x, y or z directions can be written down explicitly during the intervals in which $\alpha(t)$ is positive or negative. Thus, the phase space location at some time t for, say, the z motion, $[z(t), \dot{z}(t)/(2\alpha_0)^{1/2}]$, can be expressed as a product of matrices multiplying the initial location $[z(0), \dot{z}(0)/(2\alpha_0)^{1/2}]$. For each interval with $\alpha < 0$ or $\alpha > 0$ there is a matrix factor

$$\begin{pmatrix} \cos(\psi) & \sin(\psi) \\ -\sin(\psi) & \cos(\psi) \end{pmatrix} \text{ or } \begin{pmatrix} \cosh(\psi) & \sinh(\psi) \\ \sinh(\psi) & \cosh(\psi) \end{pmatrix} \quad (3)$$

where $\psi = (\pi/\omega_e)(2\alpha_0)^{1/2}$. The axial (z) motion is stable if the two eigenvalues of the product of the matrices in equation (3) are complex. Thus, we have stability if $[\cosh(\psi) \cos(\psi)]^2 < 1$, or $\psi < 1.875$ or

$$\alpha_0 < 0.178 \omega_e^2 \quad (4)$$

This inequality implies stability in the transverse directions; that is, the rate of switching between the mirror and anti-mirror configurations must be rapid compared with the 'natural' frequency in the mirror field $(\alpha_0)^{1/2}$.

Equations (2) with $\alpha(t) = \alpha_0 \sin(\omega_e t)$ can be solved approximately in the limit where $\alpha_0 \ll \omega_e^2$ (ref. 24). Let $x = X + \xi$, where $X(t)$ is slowly varying on the timescale $1/\omega_e$, and $\xi(t)$ is rapidly varying. Notice that $X(t)$ is analogous to the guiding centre position of a charged particle in a magnetic field while ξ represents the rapid gyration. Then, $d^2\xi_x/dt^2 = -\alpha_0 \sin(\omega_e t) X_x$ or $\xi_x \approx (\alpha_0/\omega_e^2) \sin(\omega_e t) X_x$ and $d^2X_x/dt^2 \approx -(\alpha\xi_x) \approx -(1/2)(\alpha_0/\omega_e^2) X_x$, where the angular brackets denote a time average over the interval $2\pi/\omega_e$. Similar formulae describe the y and z motion. Hence, the angular frequency for the slow motion is $\Omega_x = \alpha_0/(\omega_e\sqrt{2})$, $\Omega_y = \alpha_0/(\omega_e\sqrt{2})$, or $\Omega_z = \alpha_0\sqrt{2}/\omega_e$, for the x, y or z motion. For the guiding centre motion,

$$d^2X_j/dt^2 = -\partial V_e/\partial X_j$$

where

$$V_e(X) = \frac{1}{4} \left(\frac{\alpha_0}{\omega_e} \right)^2 (X_x^2 + X_y^2 + 4X_z^2) \quad (5)$$

is an effective potential, and $j = x, y$ or z . Note that the rapid motion ξ is out of phase with the mirror field $b(t)$: during the intervals in which the mirror field is attractive, $\alpha(t) > 0$ for, say, the x motion, the x displacement is $|X_x| + |\xi_x|$, which is larger than its value, $|X_x| - |\xi_x|$, during the intervals where $\alpha(t) < 0$. Hence, the attractive force exceeds the repulsive force. Note also that although $|\xi_j/X_j| \leq \alpha_0/\omega_e^2 \ll 1$, $|d\xi_j/dt|$ is not in general small compared with $|dX_j/dt|$.

Equations (2) and (5) can readily be generalized to account for the full spatial dependence²⁵ of the magnetic field $b_z(r, z)$ of one (or more) coaxial wire loops for $b_0^2 \ll B_0^2$. In place of equation (2), we write

$$\frac{d^2x_j}{dt^2} = \alpha(t) F_j(x) \quad (6)$$

for $j = x, y$ or z . Here, $\alpha(t) = \alpha_0 \sin(\omega_e t)$ with α_0 defined as before, below equation (2), and $F_j \equiv R^2 \nabla_j \{2b_z(r, z, t)/[3b_0(t)]\}$. An approximate solution is again obtained by letting $x = X + \xi$, where $d^2\xi_j/dt^2 = \alpha(t) F_j(X)$ or $\xi_j \approx -(\alpha/\omega_e^2) F_j(X)$. The slow, guiding centre motion obeys the equation

$$\frac{d^2X_j}{dt^2} = \sum_i [\partial F_j(X)/\partial X_i] \langle \alpha \xi_i \rangle = -\partial V_e/\partial X_j \quad (7)$$

where

$$V_e(X) = (1/4) (\alpha_0/\omega_e^2) [F(X)]^2 \quad (8)$$

is the effective potential.

Figure 1 shows a contour plot of $V_e(r, z)$ obtained from equation (8) and the actual field $b_z(r, z)$ of a single circular coil of radius R . The lowest values of the effective potential are seen to occur along the z -axis, where the effective potential is

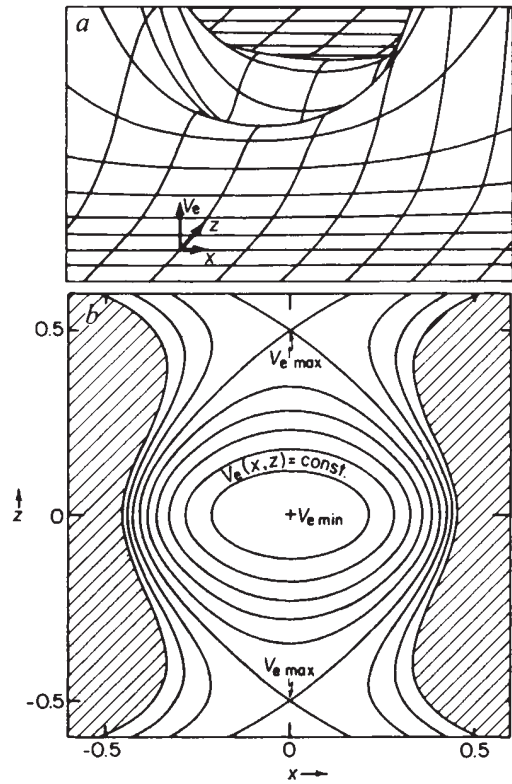


Fig. 1 Representations of the effective potential from equation (8) for the case where the b -field is caused by a single circular wire loop of unit radius in the x - y plane. *a*, An isometric view of the potential; *b*, the equipotentials. The two points denoted by V_m are separatrices. The trapped particles occupy the region $V_e \leq V_{\max} = 0.082 (\alpha_0/\omega_e)^2 R^2$. The equipotential contours are uniformly spaced with $\Delta V_e = V_{\max}/6$.

$V_e(0, z) = (\alpha_0/\omega_e)^2 z^2 [1 + (z/R)^2]^{-5}$. Thus, the 'top' of the effective potential, the separatrices of V_e , occur at $z = \pm R/2$ where $\max(V_e) \approx 0.0819 (\alpha_0 R/\omega_e)^2$.

Influence of collisions

Here, we consider the 'equilibrium' of a magnetically confined classical collisional gas in an oscillating mirror/anti-mirror field [$\alpha = \alpha_0 \sin(\omega_e t)$ in equation (6)]. Of course, the equilibrium will not be a simple Maxwell-Boltzmann distribution. For the present purposes the equilibrium can be described by the single particle distribution function (or phase space density) $f(x, v, t)$ which is a function of position x and velocity v , and which satisfies the Boltzmann equation

$$Df/Dt \equiv \partial f/\partial t + (v \cdot \nabla)f + (a \cdot \nabla_v)f = C(f, f) \quad (9)$$

where $C(f, f)$ is the Boltzmann collision integral, and $a_j = \alpha(t) F_j(x)$. A dimensionless measure of the 'strength' of the scattering is R/λ_0 . Here, $\lambda_0 \equiv [n(0)\sigma_{\text{tot}}]^{-1}$ is the mean-free path at the centre of the trap [at which point the number-density is $n(0)$], σ_{tot} is the total scattering cross-section, and R is the radius of the current loop (which is larger than the radius of the trapped gas). For the case of spin-polarized atomic hydrogen the scattering at low temperatures is predominantly the isotropic (S-wave) scattering of identical ('b' state) particles so that $\sigma_{\text{tot}} = 4\pi a_s^2$, where $a_s \approx 0.72 \times 10^{-8}$ cm (ref. 26). Thus, $\lambda_0 \approx 1.6$ cm [10^{15} cm³/n(0)].

Approximate solutions to equation (9) can be developed using the smallness of two parameters: $\varepsilon \equiv \alpha_0/\omega_e^2 \ll 1$ and $\delta \equiv \varepsilon R/\lambda_0 < 1$. We let $f = f_0 + f_1 + f_2 \dots$, where the zeroth approximation is

$$f_0(x, v, t) = K \exp(-H_0/T) \quad (10)$$

with

$$H_0(x, v, t) \equiv \frac{1}{2} [v - u(x, t)]^2 + V_e(x)$$

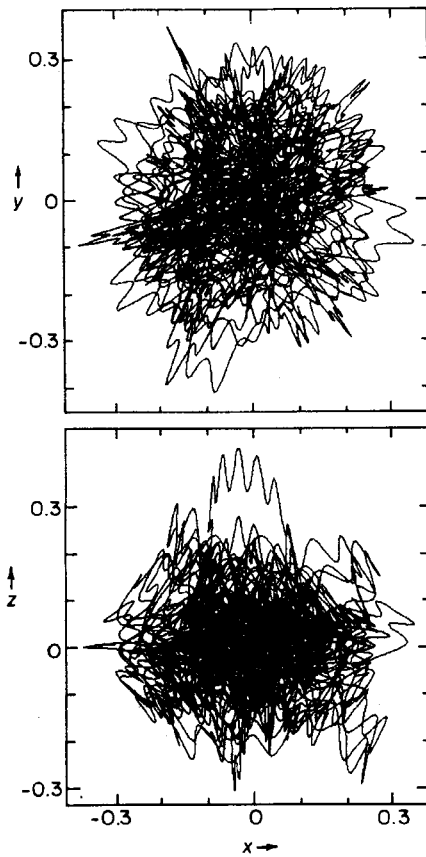


Fig. 2 Projections of the trajectory of a test particle for the conditions where $\hat{T} = 3 \times 10^{-3}$ and $\varepsilon = 0.076$. The $\mathbf{b}$ field is due to a single circular loop. This trajectory corresponds to an elapsed time of $10^3 t_e$, where $t_e = 2\pi/\omega_e$. During this interval 490 collisions occurred. A Monte-Carlo method is used to include collisions in the orbit integrations. During each time step (dt) of the fourth-order Runge-Kutta orbit integration, a random background particle velocity $\mathbf{v}_b$ is generated with v_b distributed according to equation (10). Whether or not a collision occurs is decided by a comparison of $(dt)n_b(\mathbf{x})\sigma_{\text{coll}}|\mathbf{v}_b - \mathbf{v}_t|$ with a Poisson-distributed random variable. If a collision occurs, the test particle velocity ($\mathbf{v}_t$) is modified by a rotation of $\mathbf{v}_b - \mathbf{v}_t$ in the centre of the mass frame to a randomly chosen direction. The code has been extensively tested by calculation of orbits in a time-independent potential with scattering off the corresponding Maxwell-Boltzmann background distribution $f_b(\mathbf{x}, \mathbf{v})$. The time-averaged position of the particle in phase space has been found to agree accurately with $f_b(\mathbf{x}, \mathbf{v})$, as it should.

Here, $K = \text{constant}$; $T = k_B T_0/m$, with T_0 the usual absolute temperature; V_e is given in equation (8); and $\mathbf{u} \approx d\mathbf{\xi}/dt$ is a time- and space-dependent flow-velocity with $\mathbf{\xi}$ the above-mentioned rapid component of the single-particle motion. Specifically, we take $u_j = -(d\alpha/dt)F_j(\mathbf{x})/\omega_e^2$. Therefore, $\mathbf{u}$ differs from $d\mathbf{\xi}/dt$ by a fractional amount of order ε ; and $\nabla \times \mathbf{u} = 0$ and $\nabla \cdot \mathbf{u} = 0$ in that F_j is proportional to $\nabla_j b_z$ and $\nabla^2 b_z = 0$. Thus, $\mathbf{v} = d\mathbf{X}/dt + \mathbf{u} + O(\varepsilon)$. Consequently, $H_0 = 1/2 (d\mathbf{X}/dt)^2 + V_e(\mathbf{X}) + O(\varepsilon)$ is an approximate constant of the single-particle motion. (We do not consider here the more general case where f_0 includes a dependence on the z component of the single-particle angular momentum.)

Because the collisions conserve energy and momentum, the functional form of f_0 gives $C(f_0, f_0) = 0$. This f_0 does not satisfy the Boltzmann equation (9) identically in that $Df_0/Dt = (df_0/dH_0)G$, where $G = \mathbf{v} \cdot \nabla(H_0)$. However, the time average (over intervals $2\pi/\omega_e$) of G or Df_0/Dt is zero. In order to account for the oscillating contribution from Df_0/Dt in equation (9), we let $f = f_0 + f_1$, where $Df_1/Dt = -Df_0/Dt$. Because $G = O(\varepsilon\omega_e)$, we have $f_1 = O(\varepsilon)f_0$ with $\langle f_1 \rangle = 0$. Of course, with $f = f_0 + f_1$, the collision term in equation (9) takes on the non-zero value $C(f_0, f_1) + C(f_1, f_0)$. In order to account for this contribu-

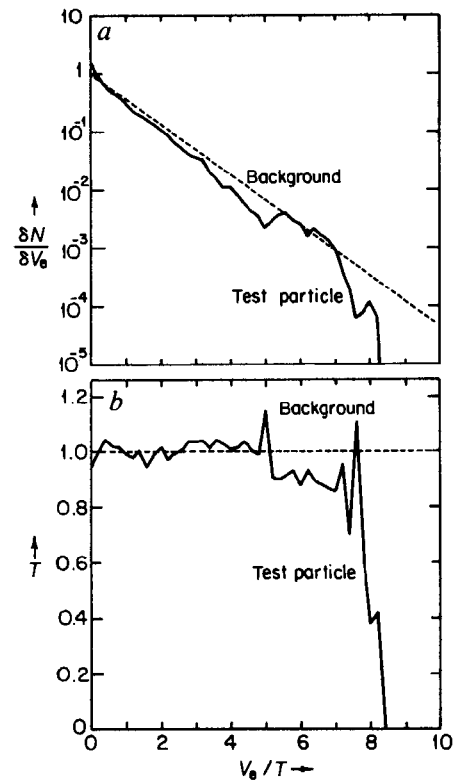


Fig. 3 *a*, The time-averaged test particle density as a function of position through its dependence on $V_e(\mathbf{x}, y, z)$. The dependence for the background is $\exp(-V_e/T)$. *b*, The dependence on V_e of the ratio of the test particle temperature ($\langle v^2 \rangle/3$) to that of the background ($T + 2V_e/3$). The test particle quantities show good approximate agreement with the theoretical background distribution function (equation (10)).

tion in equation (9), we let $f = f_0 + f_1 + f_2$, where $Df_2/Dt = C(f_0, f_1) + C(f_1, f_0)$. From this equation, we estimate $f_2 = O(\varepsilon^2 |x|/\lambda_0) f_0 < O(\varepsilon\delta) f_0 \ll f_0$.

We have numerically determined the orbits of test particles in an oscillating mirror/anti-mirror magnetic field including, via a Monte-Carlo method, collisions (S-wave scattering) of the test particle from the background distribution function given by equation (10). For a discussion of the numerical results it is convenient to use dimensionless variables: $\hat{x} = x/R$, where R is a characteristic length; $\hat{t} = t/t_e$, where $t_e \equiv 2\pi/\omega_e$; $\varepsilon = \alpha_0/\omega_e^2$; $\hat{\lambda}_0 = \lambda_0/R$, where λ_0 is the mean free path at $x = 0$; $\hat{T} = (k_B T_0/m)(t_e/R)^2$ is the dimensionless temperature; and $\hat{V}_e = (t_e/R)^2 V_e$ is the dimensionless effective potential. Figure 2 shows two projections of the test particle trajectory for an illustrative case. Notice that the axial 'width' of the orbit is approximately one-half of the transverse (x or y) width. The approximate effective potential, equation (5), implies that $\langle z^2 \rangle = (1/2)(\omega_e/\alpha_0)^2 T$ and $\langle x^2 \rangle = \langle y^2 \rangle = 2(\omega_e/\alpha_0)^2 T$. Figure 3 shows the time-averaged density and temperature as a function of position [through $V_e(\mathbf{x}, y, z)$] implied by the test particle orbit for the same case as Fig. 2.

From a large number of Monte-Carlo orbit integrations, we find that test particles are confined (for intervals $> 10^3 t_e$) only within a restricted region of the $(\hat{T}, \varepsilon)$ plane. The oscillating field $\mathbf{b}$ is considered to be that of a single circular coil of radius R . The confinement region of the $(\hat{T}, \varepsilon)$ plane is bounded from above the horizontal line $\varepsilon = \alpha_0/\omega_e^2 \approx 0.19$. Also, the confinement region is bounded from below by the diagonal line $\varepsilon \approx 1.27 (\hat{T})^{1/2}$. This condition arises from the fact that the size of the trapped gas increases with T (for fixed α_0 , ω_e and R) so that at a large enough value of T the gas escapes readily. In terms of dimensionless variables, the above-mentioned maximum of the effective potential is $\max(\hat{V}_e) = 3.23 \varepsilon^2$. Thus, the diagonal line corresponds to $T/\max(V_e) \approx 0.19$.

In terms of the physical variables, the conditions for confinement can be expressed as

$$I < 1,400 \text{ A-turn} \left(\frac{R}{1 \text{ cm}} \right)^3 \left(\frac{f_c}{10^3 \text{ Hz}} \right)^2 \quad (11)$$

and

$$I > 2,700 \text{ A-turn} \left(\frac{T_0}{1 \text{ mK}} \right)^{1/2} \left(\frac{R}{1 \text{ cm}} \right)^2 \left(\frac{f_c}{10^3 \text{ Hz}} \right) \quad (12)$$

where I is the number of Ampere-turns of the circular coil and $f_c = \omega_c/2\pi$. For a gas of atoms of mass M different from that of atomic hydrogen, the right-hand sides of inequalities (11) and (12) are multiplied by M/m and $(M/m)^{1/2}$, respectively. In order for equations (11) and (12) to be compatible we need

$$f_c > 1.9 \text{ k Hz} \left(\frac{T_0}{1 \text{ mK}} \right)^{1/2} \left(\frac{1 \text{ cm}}{R} \right) \quad (13)$$

Some illustrative values of the parameters are shown in Table 1b.

Evaporation and viscous heating

A small but finite rate of escape or evaporation of gas atoms from the magnetic trap is expected even when inequalities (11) and (12) are satisfied. We first consider the collisionless limit where $\lambda_0 \ll R$ and where an approximate treatment can be made following Jean's theory of the escape of atoms or molecules from planetary atmospheres²⁷. Clearly, from Fig. 1, the 'easiest' route of escape is along the $\pm z$ directions. We let the effective potential maximum be $V_m \equiv \max[V_c(0, z)]$ and z_m is the axial distance to the maximum. At a distance $z_c < z_m$ where the potential is $V_c = V_c(0, z_c)$, the gas effectively becomes collisionless. Out of a Maxwellian distribution of guiding centre velocities $[f(X, \dot{X})]$ of equation (10) at z_c , the atoms with outwardly directed velocities greater than the escape speed $[2(V_m - V_c)]^{1/2}$ exit from the trap. The rate of evaporation from the trap can be expressed as

$$\frac{dN}{dt} \sim -(\epsilon\omega_c)\phi N \left(\frac{V_m}{T} \right) \exp\left(-\frac{V_m}{T}\right) \quad (14)$$

Here, N is the total number of atoms in the trap, and $\phi \sim 0.07$ is dimensionless. In the collisionless limit ($\lambda_0 \gg R$), we estimate $\phi \sim 0.8 N(\sigma_{\text{tot}}/R^2)(V_m/T)$. The crossover between the two limits is at $N \sim 0.1 (R^2/\sigma_{\text{tot}})(T/V_m)$ which is $\sim 2 \times 10^{13}$ for $T/V_m = 0.15$.

The rate of energy loss of the trapped atoms due to the evaporation can be estimated using the fact that most of the escaping particles only just get over V_m . This implies a total energy loss rate of $dE/dt = 3V_m(dN/dt)$. The factor of three accounts for the fact that an escaping atom takes with it both the potential energy of its slow motion, V_m , as well as the kinetic and potential energy of its fast, 'gyratory' motion, $1/2[u(0, z_m, t)]^2 + V_c(0, z_m) = 2V_m$. Of course, the total energy of the trapped gas can be written as $E = N(K + V)$, where $\langle K \rangle$ and $\langle V \rangle$ are the average particle kinetic and potential energies, respectively. The kinetic energy is the sum of a contribution due to the slow motion (K_s) and a contribution due to the gyratory motion (K_g) and similarly for the potential energy. Because of inequality (12), most of the atoms in the trap are near the bottom of the potential well which is quadratic. Therefore, we have $\langle K_s \rangle = 3T/2$, $\langle K_g \rangle = 3T/2$, $\langle V_s \rangle = 3T/2$ and $\langle V_g \rangle = 3T/2$ so that $E = 6NT$. If evaporation were the only process affecting the total energy of the trapped gas, we would have $d(NT)/dt = (1/2)V_m(dN/dt)$ or $N[(1/2)V_m - T] = \text{constant}$. Note that our inequality (12) corresponds to $T < 0.19 V_m$.

The 'equilibrium' distribution function (10) has the non-zero flow velocity $u(x, t)$. Therefore, there is in general an associated viscous dissipation of the flow energy into heat in the trapped gas. This heating, denoted $(dE/dt)_v$, reflects the fact that a dynamic magnetic trap is not a closed system; the oscillating magnetic field does work on the gas. Because $\nabla \times u = 0$ and

$\nabla \cdot u = 0$, we have

$$\left(\frac{dE}{dt} \right)_v = 2 \int d^3x n(x) \nu(x) \left\langle \left(\frac{\partial u_x}{\partial x} \right)^2 + \left(\frac{\partial u_y}{\partial y} \right)^2 + \left(\frac{\partial u_z}{\partial z} \right)^2 \right\rangle \quad (15)$$

(ref. 28). Here ν is the kinematic viscosity which may be estimated as $\nu \sim \langle (\Delta X)^2 \rangle / \tau$, where τ is the collision time, and ΔX is the displacement of the guiding centre per collision²⁹. If $\tau \ll 2\pi/\omega_c$, the displacement per collision is of the order of the mean free path λ and the usual kinetic theory formula $\nu \sim \lambda^2/\tau$ holds. However, we expect to have $\tau > 2\pi/\omega_c$ or $\tau \gg 2\pi/\omega_c$ in most of the volume of the trapped gas. In this limit the displacement per collision is of the order of the 'gyro-radius' ξ so that $\nu \sim \langle \xi^2 \rangle / \tau$. Using this estimate, equation (15) can be integrated to give

$$\left(\frac{dE}{dt} \right)_v \sim \frac{18}{(2\pi)^{1/2}} \left(\frac{T^{1/2}}{\lambda_0} \right) \epsilon^2 NT \quad (16)$$

Here, $\lambda_0 = [n(0)\sigma_{\text{tot}}]^{-1}$, so that $\lambda_0/T^{1/2}$ is roughly the collision time at the centre of the trap. Equation (16) is in approximate agreement with the heating seen in our Monte-Carlo orbit calculations.

The combined classical influences of viscous heating and evaporative cooling are described approximately by the equations

$$\frac{dT}{dt} \sim (\epsilon\omega_c) V_m [g\epsilon^2(\sigma_{\text{tot}}/R^2)N - \phi(\frac{1}{2} - T')F(T')] \quad (17)$$

$$\frac{dN}{dt} \sim -(\epsilon\omega_c)\phi NF(T') \quad (18)$$

which follow from equations (14) and (16). Here, $T' = T/V_m$; $F = (T')^{-1} \exp(-1/T')$ and $g = 0.082(\alpha_0 R/\omega_c)^2/V_m$ with $g = 1$ for the case of a circular wire loop of radius R . If ϵ and ω_c are assumed to be constant, then from a high initial temperature, the trapped gas first moves comparatively rapidly towards the (T, N) curve along which $dT/dt \approx 0$. Along this curve, $|dT/dt|$ is small compared with the magnitude of the separate terms on the right-hand side of equation (17) which corresponds to an approximate balance of the viscous heating and evaporative cooling. Subsequently, the gas moves slowly down the $dT/dt \approx 0$ curve with the number of gas atoms decreasing gradually. The curve $a-c$ in Fig. 4 shows a typical cooling track. The viscous heating prevents the trapped gas from freely evolving into the region where Bose-Einstein condensation is expected to occur.

A possible strategy for reaching the Bose-Einstein region in Fig. 4 is as follows. We first alternate between cooling and filling the trap. Assume that there are initially a small number of atoms, N_i , in the trap at a 'high' temperature, T_i , so that the viscous dissipation is negligible. The starting conditions may be considered to be the point denoted by a in Fig. 4. After a short interval, the evaporative cooling brings the gas to point b , where the temperature is $\beta T_i < T_i$ and the density is $N = \gamma N_i$, where $\gamma \approx (1 - \tau_i)/(1 - \beta\tau_i) < 1$, with $\tau_i = 2T_i/(V_m)$. The fact that the temperature is reduced facilitates the addition of gas to the trap. This addition may be accomplished by rapidly injecting a tight clump of atoms while the effective potential barrier V_m is momentarily reduced. A sequence of cooling and filling cycles is shown in Fig. 4.

After a sufficiently large value of N is achieved (point f in Fig. 4), it becomes advantageous to do the evaporative cooling with the mirror field strength ϵ slowly decreasing with time, which has the effect of lowering the walls of the trap. This value of N is below the $dT/dt \approx 0$ curve so that viscous heating is negligible initially and its relative importance diminishes with time. In this case, the $dT/dt \approx 0$ curve in the (T, N) plane moves upward and leftward as T decreases. Because the spring constant of the effective potential well ϵ^2 is decreasing with time, we must include a term $+(T/\epsilon)(d\epsilon/dt)$ on the right-hand side of equation (17) to account for the decrease of T due to adiabatic expansion. (In the absence of evaporation T would be proportional to ϵ .) For specificity, consider programming $\epsilon(t)$ to

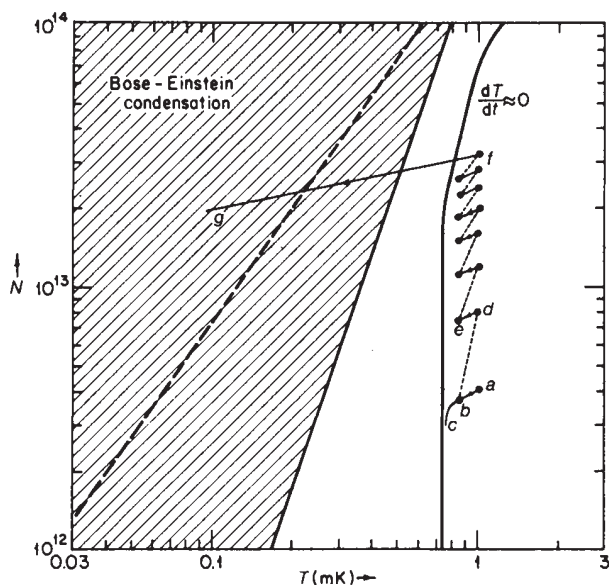


Fig. 4 Evolutionary tracks for the total number of trapped gas atoms (N) and the gas temperature (T) from equations (17) and (18) which describe the combined influences of evaporative cooling and particle loss, and of viscous heating. Except for the line f - g , the parameters are: $\epsilon = 0.06$, $T_i/V_m = 0.15$, $T_i = 1$ mK, $R = 1$ cm and $f_c = 6.9$ kHz, which are appropriate for the case where the b -field is due to a single circular loop of radius R . Along the line f - g , $\epsilon = 0.06 (T/T_i)^{1/2}$. The curve denoted $dT/dt \approx 0$ corresponds to an approximate balance of the evaporative cooling and the viscous heating. Note that equations (5) and (10) imply $n(0) \approx 1.9 (N/R^3) (V_m/T)^{3/2}$, which is $\approx 32(N/R^3)$ for $T/V_m = 0.15$. The solid straight diagonal line corresponds to $N \approx 1.7[k_B T_0 / (\epsilon \hbar \omega_c)]^3$ with $\epsilon = 0.06$, which is the condition for Bose-Einstein condensation in the static potential given by equation (5). This condition for condensation can be rewritten in terms of $n(0)$ and T so that it is similar to the formula given in the introduction. The dashed straight line is the same condition for Bose-Einstein condensation but with $\epsilon = 0.06 (T/T_i)^{1/2}$.

decrease in such a way that $T' = T/V_m = \text{constant}$. Because V_m is proportional to ϵ^2 we have $\epsilon = \epsilon_i (T/T_i)^{1/2}$, where i indicates the value at point f . From inequality (11), we must have $\epsilon_i < 0.19$. Equations (17) and (18) now imply

$$NT^{-\delta} = \text{constant} \quad (19)$$

where $\delta \equiv (\frac{1}{2})T'(\frac{1}{2} - T')^{-1}$. From inequality (11), we must have $T' < 0.19$. For $T' = 0.1, 0.15$ and 0.19 we have $\delta \approx 0.125, 0.21$ and 0.31 , respectively. The line f - g in Fig. 4 shows the evolutionary track. This track takes the gas into the region of Bose-Einstein condensation. Of course, in this region equations (17) and (18) must be modified to account for quantum mechanical effects²⁰, including, in particular, the fact that a non-negligible fraction of the trapped atoms may be in the condensate. Note that the timescale for the evolution from points a to g in Fig. 4 is quite rapid (~ 1 s). Once point g is reached, ϵ may be fixed. The evolution is then essentially frozen at point g .

Conclusions

Much effort will be needed to attain conditions for Bose-Einstein condensation of atomic hydrogen gas in a dynamic trap. The trapping region would need to be inside a thin non-conducting cylindrical wall with the coil windings for the b field located outside this wall in a large bath of liquid helium. For a coil of inner radius ~ 1 cm and, say, 2×10^3 turns, the inductance is ~ 20 mH and the currents needed are ≈ 10 A. The inductive impedance of the coil can be cancelled at the frequency f_c by an appropriate series capacitor. An estimation of the circuit resistance at resonance for copper wire gives a value $\sim 0.5 \Omega$. Thus, during the initial rapid evolution of the gas from points a to g in Fig. 4, the ohmic losses in the coil will be ≤ 25 W

during an interval ~ 1 s. Subsequently, at point g the ohmic losses will be a factor ≥ 10 smaller. The heat from the coil will go into boiling off a fraction of the helium from the bath.

A major problem will be that of supplying a sufficiently large number of low-temperature hydrogen atoms to fill the trap. The walls of the tube carrying the hydrogen atoms to the trap will be coated with a superfluid film of helium. Because the binding energy of a hydrogen atom to such a film is of the order of 1 K, the atoms will have a strong tendency to adhere to the walls for temperatures below 0.1 K. On the other hand, it is desirable to fill the trap with gas at a temperature below 0.01 K to avoid the need for even larger oscillating magnetic fields. However, it is not known how much below 0.1 K the hydrogen gas can be cooled by wall collisions. The accommodation coefficient is predicted to decrease monotonically with decreasing temperature³⁰, which means that the thermal disequilibrium between the wall and the gas becomes larger at lower wall temperatures when one is attempting to cool a 'hot' beam of atoms with wall collisions. An alternative approach may be to rely on hydrogen atom surface transport via diffusion along the helium film surface to the trap region (J. D. Reppy, personal communication). It may be possible to detach the hydrogen atoms from the film surface in the trap region using carefully controlled thermal³¹, sonic or magnetic gradient pulses. Another approach might involve forming a collimated and velocity-selected atomic beam which is then slowed down by a magnetic field gradient.

Another important challenge will be the detection of the Bose-Einstein condensation. Sensitive techniques are available for detecting hydrogen atoms using nuclear magnetic resonance (where 10^{14} atoms can easily be detected)³² and electron spin resonance (where 10^9 atoms can be detected)^{33,34}. In the potential well provided by the trap, the condensed hydrogen atoms (which are bosons) will tend to congregate in the low-energy states of the well (energies $\ll k_B T$), which are relatively compact in configuration space^{20,35-37}. A clear manifestation of Bose-Einstein condensation would be the appearance of a spatially compressed (condensate) component of the trapped gas. This compressed component should be evident in magnetic resonance imaging experiments. Another remarkable prediction³⁸ is that of the appearance and precession of a spontaneous magnetization of the condensate of a hydrogen gas made up of both 'a' and 'b' states. It seems reasonable to expect that various new quantum phenomena will be found in Bose-Einstein condensed atomic hydrogen gas.

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An inherited limb deformity created by insertional mutagenesis in a transgenic mouse

R. P. Woychik*, T. A. Stewart*, L. G. Davis*, P. D'Eustachio† & P. Leder*

* Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA

† Biochemistry Department and Kaplan Cancer Center, New York University Medical Center, New York, New York 10016, USA

We have created an insertional mutation that leads to a severe defect in the pattern of limb formation in the developing mouse. The novel recessive mutation is phenotypically identical and non-complementary to two previously encountered limb deformity mutations, and is closely linked to a dominant mutation that gives rise to a related limb dysmorphism. The inserted element thus provides a molecular genetic link with the control of pattern formation in the mammalian embryo.

THE molecular events that guide pattern formation during embryonic development have been difficult to characterize in mammals, partly because of the difficulty in drawing a molecular connection between the phenotype of a dysmorphic mutation and the specific gene in which that mutation occurs. One means of overcoming this problem involves creating developmental mutants by insertional mutagenesis in transgenic mice and using the inserted DNA fragments as markers to clone the mutant genes. Initially, one might expect such mutants to be prohibitively rare, but, using a related approach, Jaenisch and colleagues^{1,2} have identified an embryonic lethal mutant as an insertional mutation in the $\alpha 1(I)$ collagen gene. Additional potentially interesting mutants in transgenic mice have been identified by others^{3,4}.

Recently, we created several strains of transgenic mice for experiments designed to understand the action of the oncogene *c-myc*⁵. To test for gene dosage effects, these strains were bred to homozygosity with respect to the inserted gene (in this case, a fusion construct of the mouse mammary tumour virus long terminal repeat and the mouse *c-myc* gene; MMTV-*myc*). We also systematically screened these animals for recessive lethal or dysmorphic mutations. Among seven transgenic mouse strains analysed in this way, we discovered one carrying a recessive mutation that results in a severe dysmorphism of all four limbs.

Discovery of limb deformity mutation

In preparing transgenic mice, the DNA fragments which are injected into the fertilized egg integrate into the host genome, usually at a single site, and become a stably heritable genetic element⁶⁻¹¹. From the analyses that have been done so far, no clear rules have emerged regarding the site of integration. It is, therefore, conceivable that these DNA fragments will occasionally integrate within or near, and hence disrupt the function of, a gene whose expression is essential for the normal development of the animal. For this and other reasons related to our original experimental purposes, we systematically intercrossed transgenic heterozygous animals. In this manner, the phenotype of any recessive mutation caused by the inserted MMTV-*myc*

sequences would become apparent on examination of the homozygous offspring.

In one of these lines of transgenic mice, designated the S line (previously called 165-1a)⁵, some of the offspring from heterozygous parents were abnormal. The fore- and hindlimbs of these animals were deformed, and some of the digits in their fore and hind paws appeared to be fused (Fig. 1A). We analysed the skeletons of several affected animals and noted that in all cases there was a synostosis of the long bones (radius-ulna/tibia-fibula), oligodactyly (absence of post-axial digits), fusion of some carpals, tarsals, metacarpals and metatarsals, and syndactyly (Fig. 1B). The axial skeleton appeared normal. To enhance their chances of survival, the affected animals usually had to be removed from the competition of their wild-type littermates during nursing. As adults, at least some of the affected animals have proven to be fertile.

Co-segregation

To investigate whether the mutation causing the limb deformity in these transgenic animals co-segregated with the MMTV-*myc* integration site, several mice known to be heterozygous for the limb deformity were mated either with each other or with homozygous wild-type littermates (Table 1). The latter matings

Table 1 Limb deformity phenotype segregates as a recessive trait

No. of different parents used*	Parent†				
	♂ × □	○ × ▣	♂ × ▣	● × □	○ × ▣
	5 2	3 2	15 8	1 1	1 1
No. of litters	5	5	22	1	1
Total no. of pups	31	26	186	10	7
No. of pups displaying a limb deformity phenotype	0	0	39	0	0
Per cent limb deformity phenotype	0	0	21	0	0

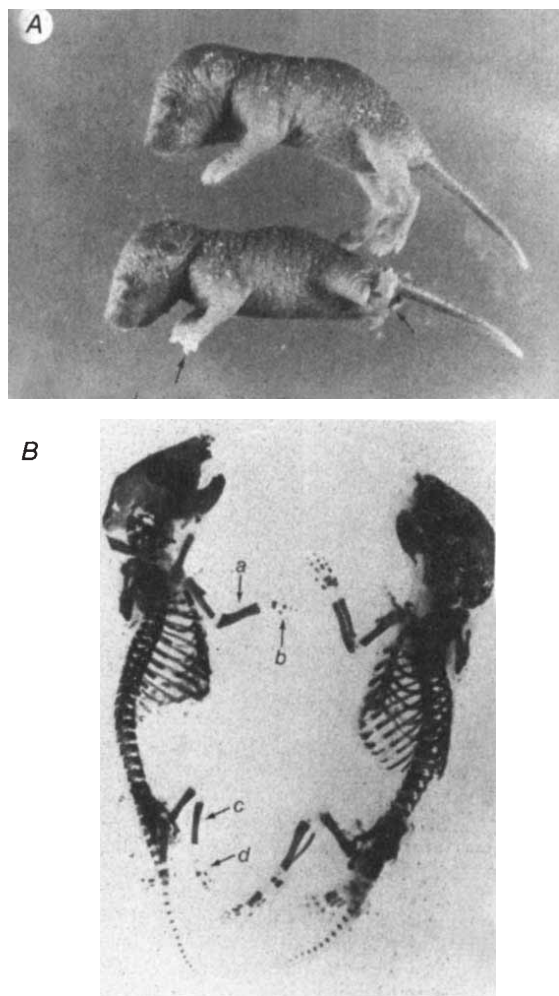
* Refers to the number of different individual wild-type, heterozygous or homozygous animals which were used to generate the numbers within each column of data.

† Circles represent females, squares males.

† Present addresses: Central Research and Development Department, Experimental Station, E. I. DuPont de Nemours & Co., Inc., Wilmington, Delaware 19898, USA (L.G.D.); Genentech, 460 Point San Bruno Boulevard, South San Francisco, California 94080, USA (T.A.S.).

Fig. 1 Characteristics of the limb deformity phenotype. The figure shows a mouse expressing the limb deformity defect compared with a normal littermate. **A**, Two 4-day-old animals from the offspring of a mating between heterozygous parents. The lower animal expressed the mutant phenotype; arrows indicate the defective fore- and hindlimbs. **B**, Skeletal structures of the homozygous mutant (on the left) and the wild-type mouse. The abnormal regions of the mutant skeleton are indicated by arrows: *a*, synostosis of the radius and ulna; *b*, oligodactyly and syndactyly of the digits of the fore paw, as well as fusion of some carpals and metacarpals; *c*, synostosis of the tibia and fibula; *d*, oligodactyly and syndactyly of the digits in the hind paw, as well as fusion of some tarsals and metatarsals.

Methods. To visualize the skeleton of the animals, the staining procedure described by McLeod²⁴ was used. Briefly, the animals were eviscerated and their pelage was removed. The samples were then placed in 95% ethanol for 7 days, followed by 24 h in acetone. The bones were stained red and the cartilage stained blue in a solution containing Alizarin red (Sigma) and Alcian blue (Sigma). The soft tissue was then cleared in a 1% KOH solution to allow the bone and cartilage to be visualized.



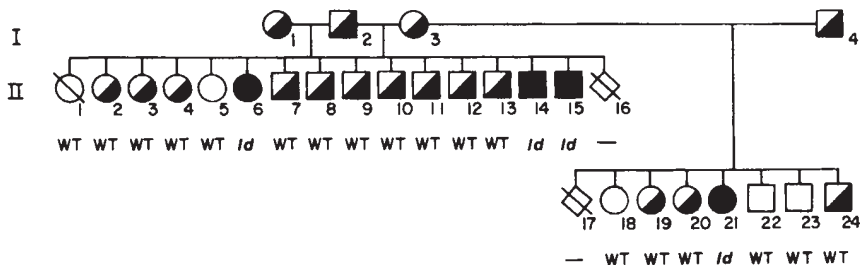
(heterozygote $\times$ homozygous wild type) yielded no animals with the limb deformity among 57 offspring from 10 litters. Similarly, matings between homozygous affected and wild-type littermates yielded no affected offspring. The cross between heterozygous individuals, however, yielded 21% deformed animals among 186 offspring from 22 litters. These results are consistent with an autosomal recessive mode of inheritance at a single locus (39 affected individuals among 186 informative offspring: 46.5 were expected to be affected, $\chi^2_1 = 1.612$; $0.10 < P < 0.25$). To test the possibility that the limb abnormality was co-segregating with the MMTV-*myc* sequences, genomic DNAs from the tails of individuals from three representative litters were analysed to determine their genotypes (using the restriction fragment length polymorphism (RFLP) analysis described in Fig. 3 legend) (see Fig. 2). Only individuals homozygous for MMTV-*myc* sequences expressed the limb abnormality; MMTV-*myc* heterozygotes and animals entirely lacking these sequences were phenotypi-

cally normal. The recessive limb deformity thus appears to segregate with the site at which the MMTV-*myc* sequences integrated. Hereafter, *ld*^{hd} will be used to refer to this mutant locus.

Cloning of mouse genomic DNA

As the MMTV-*myc* sequences segregated with the limb abnormality, the mutation giving rise to the defect may have been caused by the integration of the foreign DNA sequences. While the disorder might be the result of expression of an inserted gene, it is more likely (see below) that the MMTV-*myc* sequences integrated into, and hence disrupted the function of, a cellular gene that participates in the development of the fore- and hindlimbs during embryogenesis. To evaluate this possibility, we cloned the mouse genomic DNA flanking both sides of the inserted MMTV-*myc* sequences in the genome of the transgenic animal.

Fig. 2 A representative pedigree showing offspring of heterozygous parent mice from the S transgenic line. The offspring (II-1 to II-24) of two different females (I-1 and I-3) and two different males (I-2 and I-4) are shown. The offspring numbered II-1 to II-16 represent a combined litter of animals from the mating of the I-1 and I-3 females with the I-2 male. All four parents were phenotypically normal; the phenotype of the individual animals is indicated by WT for wild type and *ld* for limb deformity. Males are represented by squares, and females by circles. Individuals that do not contain the MMTV-*myc* sequences are indicated by open circles or squares. Half-filled circles or squares represent transgenic heterozygotes, while completely filled circles or squares represent homozygous transgenic animals. Heterozygosity was distinguished from homozygosity using the RFLP method indicated in Fig. 3 legend. $\diamond$, The animal died before its sex could be determined. A slash through a symbol indicates that the animal died before its genotype could be established.



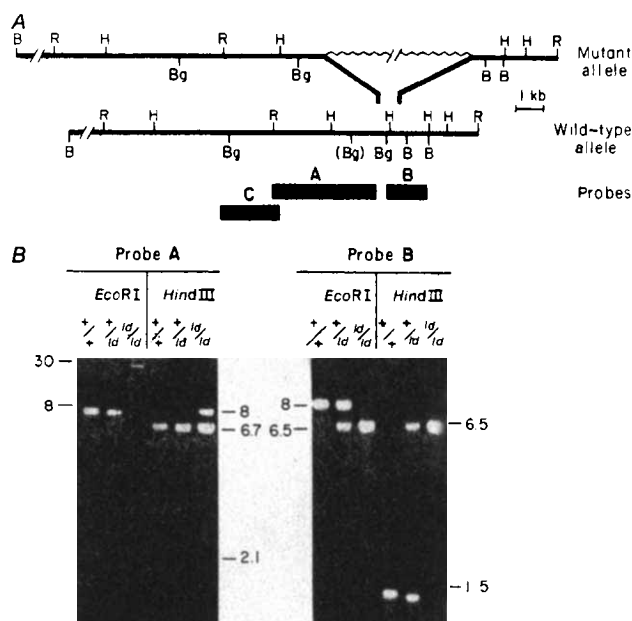


Fig. 3 Characterization of the region of the mouse genome interrupted by the inserted MMTV-myc sequences. **A**, Structure of a region of the mutant mouse allele containing the inserted MMTV-myc sequences (indicated by a wavy line) and its corresponding normal allele. The sequence between the bold lines which extends down from the mutant allele to specific sites on the normal allele is deleted in the mutant allele. Three different DNA fragments (A, B and C), represented by solid boxes, were nick-translated and used as probes in the present study. Restriction enzyme sites: H, *HindIII*; R, *EcoRI*; B, *BamHI*; Bg, *BglII*. (The *BglII* site in parentheses is a polymorphic site and is present in only some of the alleles at this locus.) **B**, Southern blot of *EcoRI*- or *HindIII*-digested genomic DNA from an outbred CD1 mouse (+/+) or from heterozygote (*ld*/+) or homozygote (*ld/ld*) transgenic mice. (In this figure *ld* refers to the *ld^{Hd}* mutation.) The samples were hybridized with the nick-translated restriction fragments indicated as probes A and B in **A**. Numbers refer to the size (in kb) of the hybridizing fragments.

Methods. A portion of the mutant allele was initially cloned by preparing a genomic cosmid library with size-fractionated (30–40 kb), partial *Sau3A*-digested genomic DNA fragments from the liver of an animal of the S transgenic line. These fragments were ligated into a cosmid vector with an inactivated ampicillin resistance (*Amp^r*) gene. The cosmid vector that we used for this purpose was derived from the c2RB vector constructed by Bates and Swift²⁵ which contains both *Amp^r* and kanamycin resistance (*Kan^r*) genes. The *Amp^r* gene in the c2RB vector was inactivated by digestion at the unique *PvuI* site in its *Amp^r* gene, filling-in the overhanging single-stranded ends with T4 DNA polymerase, and then blunt-end ligating the molecule with T4 DNA ligase. The *Kan^r* gene between the *cos* sites facilitated the growth of the modified vector in *Escherichia coli*. Cosmids which grew on ampicillin plates had acquired an *Amp^r* gene from the pBR322 sequence in the transgenic mouse DNA. (These pBR322 molecules were injected together with the MMTV-myc sequences in the preparation of the S line transgenic mice.) One such cosmid contained an insert comprised of a segment of MMTV-myc sequence together with 9 kb of mouse genomic sequence. The wild-type allele was cloned by screening a normal mouse library²⁶ which we prepared using the unmodified c2RB vector and a probe derived from the 9 kb of mouse genomic sequence that was cloned with the *Amp^r*-defective vector. Only a portion of the cloned sequences is shown in the figure.

We initially cloned 9 kilobases (kb) of mouse genomic DNA from the region flanking one side of the MMTV-myc sequence using a cosmid vector that was specifically modified for this experiment. This vector allowed us to select for the β -lactamase activity on the pBR322 sequences that were interspersed with the MMTV-myc sequences in the genomic DNA of the transgenic animal (see Fig. 3 legend for details). A region of this cloned 9-kb sequence was then used as a probe to isolate the

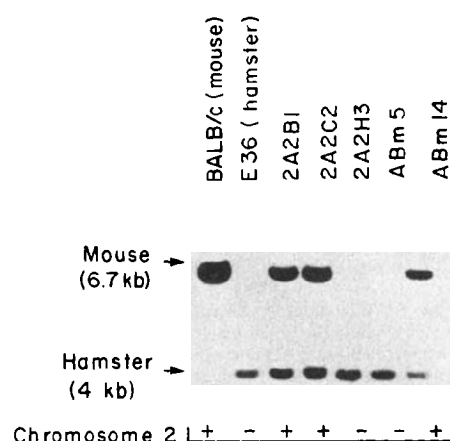


Fig. 4 The MMTV-myc integration site lies on chromosome 2. Using the Southern blot technique²⁷, we analysed wild-type BALB/c mouse and E36 hamster DNA samples, as well as DNA extracted from the 2A2B1, 2A2C2, 2A2H3 (ref. 22) and ABm5 and ABm14 (ref. 23) mouse-hamster somatic cell hybrids. A detailed chromosomal analysis of these somatic cell hybrids is given in Table 2. Genomic DNA (20 μ g) was digested with *HindIII*, electrophoresed through a 0.8% agarose gel, then transferred to nitrocellulose²⁷. A nick-translated restriction fragment, designated probe C in Fig. 3, was used as a probe for this experiment. The numbers refer to the size of the mouse and hamster hybridizing fragments. The presence or absence of mouse chromosome 2 in the individual samples of DNA is indicated by a + or -.

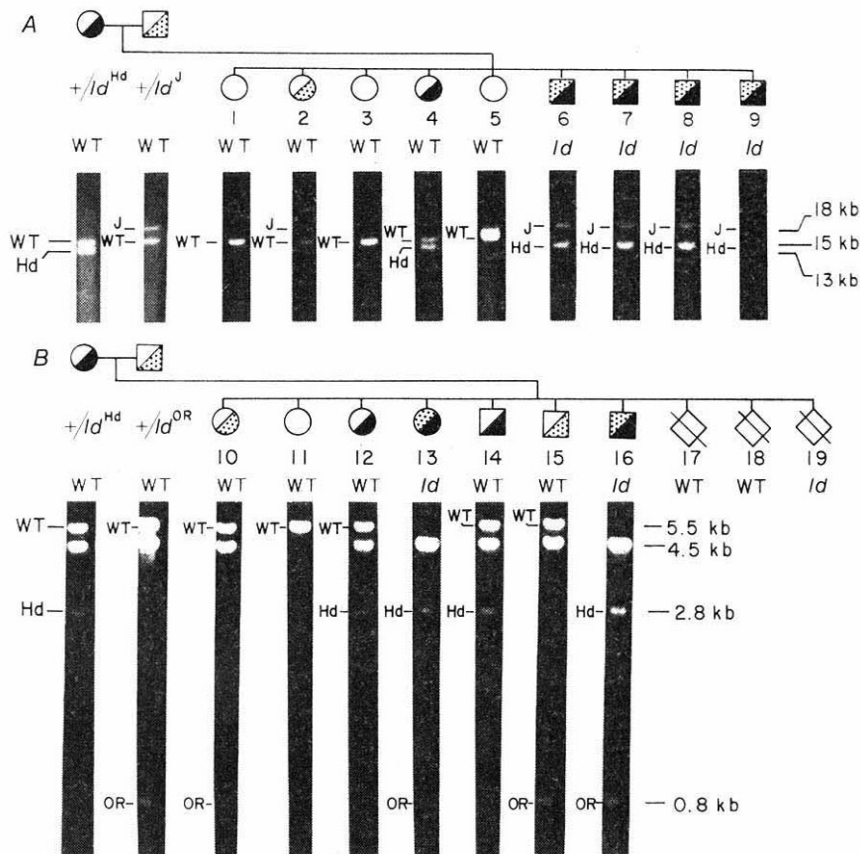
uninterrupted copy of this locus from a cosmid library constructed with DNA from a wild-type mouse. Using these clones, the structure of the locus into which the MMTV-myc sequences integrated was deduced (Fig. 3A).

To determine the structure of the interrupted mutant allele, genomic DNAs from a wild-type mouse (+/+) and from transgenic mice containing either one copy (*ld^{Hd}/+*) or two copies (*ld^{Hd}/ld^{Hd}*) of the interrupted allele were digested with *EcoRI* or *HindIII* and hybridized with sequences from either side of the insertion site (probes A and B in Fig. 3A). Both probes hybridized to the 8-kb *EcoRI* fragment that spans the insertion site in the wild-type allele (see Fig. 3B). In contrast, probe A hybridized to a 30-kb fragment and probe B hybridized to a 6.5-kb fragment in the mutant allele. The size-altered hybridizing fragments from the mutant allele probably represent the junction restriction fragments that contain a portion of the inserted MMTV-myc sequences. With the *HindIII*-digested DNA, probe A hybridized to 2.1-kb and 6.7-kb fragments on the wild-type sample; an 8-kb fragment was observed instead of the 2.1-kb fragment in the mutant allele. Using probe B and *HindIII*-digested DNA, a 1.5-kb hybridizing fragment (corresponding to the restriction fragment used as a probe for this analysis) was observed with the wild-type DNA; this fragment was replaced by a 6.5-kb fragment in the mutant allele. Based on these results, as well as other data including similar analyses with several other restriction enzymes (data not shown), it appears that a small region of ~1 kb has been deleted from the mouse genome as a consequence of the integration of the MMTV-myc sequences. Aside from this deletion, no other gross rearrangement within several kilobases of the integration site has been detected (data not shown).

Chromosomal mapping of integration site

Several morphogenetic loci that affect the development of the fore- and hindlimbs of the mouse have been identified¹². To determine whether the site of insertion of the MMTV-myc sequences correlates with the position of any of these loci, we chromosomally mapped the insertion segment. For this purpose, we hybridized genomic DNA from several mouse-hamster somatic cell hybrids with a probe derived from a region near the site of integration (Fig. 4). Based on the results of this

Fig. 5 Pedigree showing the 19 offspring of the mating of heterozygous parents containing the ld^{Hd} , ld^J or ld^{OR} limb deformity alleles. The phenotypes of the parents and offspring are indicated: WT, wild-type; ld , limb deformity. Symbols are as described for Fig. 2, except that $\square$, $\circ$, $\otimes$ and $\boxtimes$ indicate that the animal was a heterozygote. Circles or squares which are both half-solid and half-stippled refer to those animals that had inherited two defective copies of the limb deformity allele, one from each parent. **A**, Analysis of the nine offspring (1-9) from a single mating between a heterozygous S line transgenic ($ld^{Hd}/+$) mother and a Jackson ($ld^J/+$) father. The wild-type allele migrates as a 15-kb fragment, while the mutant transgenic and Jackson alleles migrate as 13- and 18-kb fragments, respectively. **B**, Analysis of the 10 offspring (10-19) from a single mating between a heterozygous S transgenic ($ld^{Hd}/+$) mother and an Oak Ridge ($ld^{OR}/+$) father. The wild-type allele migrates as a 5.5-kb fragment, while the ld^{Hd} and ld^{OR} alleles migrate as 2.8- and 0.8-kb fragments, respectively. (The 4.5-kb fragment is present on both the ld^{Hd} and ld^{OR} alleles, but not on the wild-type allele; this is due to the presence of the polymorphic *Bgl*II site (shown in parentheses in Fig. 3A) in both mutant alleles). In each case 10 μ g of genomic DNA from the parents or from their individual offspring were digested with *Bam*HI (**A**) or *Bgl*II (**B**), electrophoresed on a 0.8% agarose gel, transferred to nitrocellulose²⁷, then hybridized with a nick-translated restriction fragment (probe A in Fig. 3).



analysis, we have assigned the insertion site to chromosome 2, distal to band C1. The murine 6.7-kb hybridizing fragment was detected only in those somatic cell hybrids containing chromosome 2 or a derivative of chromosome 2 distal to band C1 (Table 2, Fig. 4). The hamster DNA yielded a 4-kb hybridizing band of moderate intensity, even under the high-stringency washing conditions used in the analysis ($0.2\times$ SSC, 0.1% SDS, 65°C); this fragment in the hamster genome must contain a region that is highly homologous with a section of the mouse restriction fragment used here as a probe.

Complementation tests

Examination of the genetic map of mouse chromosome 2 revealed the presence of several mutations which affect the development of the fore- and hindlimbs¹³. In particular, one mutation (designated ld for limb deformity) was reported to have an inheritance pattern and mutant phenotype strikingly similar to those of our transgenic mice. As we had observed with our mutant mice, in animals expressing this ld mutation the tibia and fibula were replaced by a single bone, the radius

and ulna were fused, and the bones in the fore and hind paws were reduced¹⁴⁻¹⁶. Two alleles of this mutation have thus far been observed: one at Jackson Laboratories (designated here as ld^J) and the other, possibly radiation-induced, at Oak Ridge National Laboratories (designated here as ld^{OR}). Both these mutations have been preserved by over 20 years of continuous passage.

To test for complementation between the ld^{Hd} mutation in our transgenic mice and the ld^J and ld^{OR} mutations, female transgenic heterozygotes ($ld^{Hd}/+$) were mated with heterozygous male animals from either the Jackson ($ld^J/+$) or Oak Ridge ($ld^{OR}/+$) laboratories (Fig. 5). As all three limb deformity mutations are recessive, offspring expressing the characteristic limb deformity should occur only if the mutations occur in the same complementation group. Examination of the offspring from these matings revealed several individuals expressing a limb defect that was identical to that which we observed in the transgenic mice (Fig. 5). This finding suggested that the ld^{Hd} mutation might be allelic to the ld^J and ld^{OR} mutations.

To investigate this point further, we identified RFLPs that

Table 2 Chromosomal analysis of the mouse-hamster somatic cell hybrids used in experiment of Fig. 4

Somatic cell hybrid	Mouse chromosomes present																			Reaction with mouse probe*
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
2A2B1	+	+	+	+	-	+	+	+	+	+	-	+	-	+	+	+	+	-	+	+
2A2C2	+	+	+	+	-	-	+	+	+	+	-	+	+	-	+	+	+	-	+	+
2A2H3	+	-	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-
ABm5	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ABm14	-	+	-	-	-	+	-	-	-	-	-	-	-	-	+	-	+	-	-	+

Metaphase spreads prepared from somatic cell hybrids were scored for the presence of mouse chromosomes by a combination of trypsin-Giemsa and Hoechst 33258 staining. + Indicates presence of a chromosome at a frequency ≥ 0.15 copies per cell, the approximate limit of detection of the Southern blotting assay^{22,23}.

* Scored by Southern blotting, as shown in Fig. 4.

† Abm5 contains only a deletion derivative chromosome 2, del2(C1→ter)²³.

segregated with the ld^J and ld^{OR} mutations. As shown in Fig. 5A, the ld^J mutation segregated with an 18-kb *Bam*HI fragment that could be distinguished from both the 13-kb fragment that segregated with the ld^{Hd} mutation and the 15-kb wild-type fragment. In addition, the ld^{OR} mutation segregated with a 0.8-kb *Bgl*II fragment, which differs in size from the 2.8-kb and 5.5-kb *Bgl*II fragments that segregated with the ld^{Hd} and wild-type genes, respectively (Fig. 5B). (With the probe used for this experiment, the 4.5-kb *Bgl*II fragment shown in Fig. 5B is common to both the ld^{OR} and ld^{Hd} mutants.) These polymorphisms allowed us to establish the ld genotype of the offspring from the matings described above (Fig. 5A, B).

A comparison of the genotype of the individual animals with their corresponding phenotype revealed that animals that inherited two copies of the wild-type RFLP or only one copy of a mutant-linked RFLP were phenotypically normal. On the other hand, those animals that inherited a mutant-linked RFLP from both parents displayed the mutant phenotype. Therefore, the mutation reported for our transgenic animals is probably allelic to the ld^J and ld^{OR} mutations.

Development of the phenotype

There are two arguments that mitigate against a direct role for the *c-myc* gene product in the development of the ld phenotype. The more compelling argument follows from the occurrence of the two previously characterized mutations that give rise to the ld phenotype. As neither mutation involves an aberrant *c-myc* at the ld locus, it is clear that the phenotype can arise independently of an aberrantly expressed *c-myc* gene. In addition, expression of the inserted *c-myc* fusion gene occurs in the mammary gland and other tissues of heterozygotic S line transgenic mice (ref. 5 and data not shown). Assuming that there are no gene dosage effects, one would expect the mutation to be dominant if expression of the MMTV-*c-myc* gene is involved in the development of the ld phenotype. Indeed, the inserted fusion gene does behave as an autosomal dominant mutation with respect to the development of breast adenocarcinomas in S strain females (data not shown). Therefore, the autosomal recessive behaviour of the mutations is most easily reconciled with loss of function of the ld gene.

Role of ld gene product

One model of ld gene action holds that the normal homologue of the gene is temporally regulated during development and is expressed within the cells comprising the developing limb bud. In the mouse, the forelimbs develop first and appear as outgrowths of tissue along the side of the developing 10-day embryo¹⁷. During the next few days, the limb buds undergo extensive proximal-distal elongation, a process that may be under the control of the apical ectodermal ridge at the distal rim of the limb bud¹⁸. It has been speculated that control of

anterior-posterior pattern formation in the limb bud (the axis affected by the ld mutation) is governed by a morphogen gradient produced by the cells in a region called the zone of polarizing activity¹⁹. As the postulated gradient produced by this zone could affect the normal development of the radius, ulna and digits²⁰, the limb deformity mutation might disrupt the function of a gene normally expressed in this zone. On the other hand, expression of the gene at the ld locus could also occur in the mesenchymal cells that ultimately condense to form the bony structures within the limb. For example, the product of the ld gene could be a growth factor, a growth factor receptor or a specific cell-surface identity marker. Hopefully, additional experiments using cloned fragments from the ld locus will allow us to identify the site(s) of synthesis and action of the ld gene product.

Potential relationship to *lst* mutation

Another limb bud mutation has been mapped within two centimorgans of the ld mutation on chromosome 2 of the mouse. The mutation, Strong's luxoid (*lst*), exhibits genetic and phenotypic behaviour that is opposite from that of ld^{21} : instead of being recessive, it is dominant and instead of giving rise to too few bones in all four limbs, it gives rise to too many, that is, it is characterized by, among other things, polydactyly and duplications of the radius and tibia. As ld and *lst* were both mapped with respect to external markers and were not tested for allelism (P. Lane, personal communication), it is possible that these mutations lie much closer to one another than is suggested by the resolution of the current mapping data. We propose a very simple model that accounts for each of the mutations by allowing them to occur in the same locus. Thus, ld could represent a class of recessive mutations resulting in loss of gene function, for example by alteration of a coding sequence or promoter site, while *lst*, with its dominant behaviour, could act as a regulatory mutation in the same gene, for example by increasing the level of the ld product or allowing it to be expressed inappropriately. Fortunately, *lst* has also been preserved for the past 30 years, most recently as a frozen embryo, and will soon be available to test this possibility.

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Randomness in quantum mechanics—nature's ultimate cryptogram?

T. Erber*

Department of Physics, University of California, Los Angeles, California 90024, USA

S. Putterman*

Department of Applied Mathematics, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, UK

Will a single atom irradiated by coherent light be equivalent to an infinite computer as regards its ability to generate random numbers? As described here, a search for unexpected patterns of order by cryptanalysis of the telegraph signal generated by the on/off time of the atom's fluorescence will provide new experimental tests of the fundamental principles of the quantum theory.

Dehmelt's pioneering work on the storage of single electrons and ions in macroscopic potential wells is currently being extended to neutral atoms and more complicated trapping schemes^{1,2}. These systems are interesting not only because they permit extremely accurate measurements of frequencies, or energies, but also because they are potentially useful in exploring new quantum effects. One possibility is the observation of individual quantum jumps by means of optical double-resonance methods^{1,3}. The novel aspects of this arrangement are that the fluorescent decays of a single atom can be observed, and further that the system can be reset by coherent excitation. The time delays between the resets and decays form a nominally 'random' binary telegraph signal. If the jumps are inherently indeterminate, arbitrarily long strings of random numbers can be generated by these signals⁴. In this respect quantum mechanics implies that even the simplest physical systems are equivalent to algorithms and computers of unbounded complexity⁵. This idea can be checked by searching for latent patterns of order in the sequence of fluorescent decay times. Powerful new theorems on the orbit structures of pseudo-random functions can aid in the cryptanalysis of these telegraph signals⁶. Externally induced correlations can also be exploited for precision field measurements.

The essential components of Dehmelt's shelved electron scheme for generating random fluorescence signals are shown in Fig. 1. A single atom or ion is stored in a macroscopic potential well. The present state of this technique is illustrated by the fact that a single Ba^+ ion can be localized to within $0.2\ \mu\text{m}$, or $2,000\ \text{\AA}$, in a radio frequency quadrupole trap with an effective well depth of $10\ \text{V}$: with a vacuum of 10^{-11} torr, or a residual gas density of 10^5 molecules cm^{-3} , the ion can be cooled to an equivalent temperature of $10\ \text{mK}$ and stored for hours^{1,7}. If the trapped object has an energy level structure of the type indicated in Fig. 1, a random telegraph signal can be generated by double-resonance methods³. Specifically, let us suppose that the ground state O and the excited level S are connected by a strong optical transition—that is, the matrix element $\langle O | e^{-i\mathbf{k}\cdot\mathbf{r}} | S \rangle$ is large. Then incident light (with wave vector $\mathbf{k}$) at frequencies matching ω_{strong} will induce transitions of an atomic electron between O and S at the Rabi flopping frequency. All the downward transitions $S \rightarrow O$ are accompanied by photon emission. A fraction of these re-radiated induced will be coherent with the incident light in virtue of emission or coherent Compton scattering. These coherent photons will be mixed with the transmitted beam. Experimentally it is much easier to detect the incoherent or fluorescent photons that are emitted transversely to the incident beam. In particular, if the strong transition $O \rightleftharpoons S$ is saturated

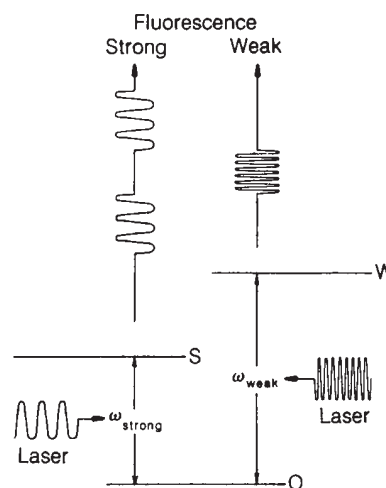


Fig. 1 Double-resonance method for generating a nominally random telegraph signal. During the time that an atomic electron is 'shelved' in state W, the fluorescence signal emanating from the $O \rightleftharpoons S$ transitions is quenched.

by the incident light, the fluorescent emission can be of the order of 10^8 photons s^{-1} , and this luminosity is sufficient to make the single trapped object visible through a microscope^{1,7}.

Suppose now, as shown in Fig. 1, that the ground state O is also weakly coupled to another excited level W. If the trapped object is simultaneously illuminated by two beams, one tuned to ω_{strong} and the other to ω_{weak} , the electron will continue to make many rapid transitions between O and S, but occasionally the incident ω_{weak} -beam will excite the upward transition $O \rightarrow W$. Clearly, while the electron is 'shelved' in state W, the fluorescence signal associated with the strong transition $S \rightarrow O$ will be shut off. However, when the electron returns to the ground state O via a spontaneous or induced decay, the strong fluorescence will switch on again. In this sense the fluorescence emanating from the strong transition is a direct indicator of the state of excitation of the weak transition³. Moreover, as the photon emission rate of the strong transition is very high, the photoelectron yield of a detector will, in first approximation, be equivalent to that generated by a steady beam of light with intensity I_0 ⁸. Similar couplings of quantum jumps to classical current pulses occur in SQUID analogue-to-digital converters⁹. The quantum jumps of the shelved electron may then be used to generate the telegraph signal shown in Fig. 2: the time intervals during which the signal is turned off (t_1, t_2 , etc.) indicate when the electron occupies state W; the 'on' periods (T_1, T_2 , etc.) correspond to the rapid transitions $O \rightleftharpoons S$ that occur between the weak excitations of the electron from O to W.

In general, the time intervals for which the signal is off are distributed randomly with an exponential weight e^{-t/t_w} , where t_w is the decay time of the weak transition. If, as usual, A_w and B_w denote the coefficients for spontaneous and induced emission for the transition between W and O, and $U(\omega_{\text{weak}})$ is the spectral energy density of the incident ω_{weak} light, then this decay time is given by³: $t_w = [A_w + B_w U(\omega_{\text{weak}})]^{-1}$. Similarly, the time intervals for which the signal is on are distributed exponentially with weight e^{-T/T_w} , where $T_w = 2[B_w U(\omega_{\text{weak}})]^{-1}$. In principle, both of these stochastic sequences can be used to generate random numbers. For example, if the time intervals are rescaled in terms of the decay time, $\tau_i \equiv t_i/t_w$, the numerical sequence $\{e^{-\tau_i}\}$ is random and uniformly distributed in the interval (0, 1). A measure of the fluctuations of the signal durations is given by the average magnitude of the difference between two arbitrarily chosen intervals, that is,

$$\langle |\tau_i - \tau_j| \rangle_{i,j} = \int_0^\infty \int_0^\infty d\tau_i d\tau_j |\tau_i - \tau_j| e^{-(\tau_i + \tau_j)} = 1 \quad (1)$$

We will shortly see that these and other statistical characteris-

* Permanent addresses: Department of Physics, Illinois Institute of Technology, Chicago, Illinois 60616, USA (T.E.); Department of Physics, University of California, Los Angeles, California 90024, USA (S.P.).

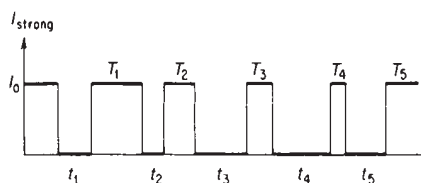


Fig. 2 Fluorescence telegraph signal. The time intervals $t_1, t_2, \dots, t_i$ during which the signal is 'off' correspond to the 'shelving' of an electron in state W; the signal is pulsed 'on' in the intervals $T_1, T_2, \dots, T_i$ due to the strong fluorescence transitions between S and O.

tics of random exponential sequences can be simulated by deterministic algorithms. Furthermore, previous experience with analogue and hybrid devices in random-number generation has shown that these sources have correlational defects⁴. Therefore, before any serious attempts can be made to adapt the fluorescence telegraphs for the generation of random signals, it will be necessary to check to what extent the experimentally observed time sequences $\{t_i\}$ exhibit patterns of order. A crucial advantage of using single trapped objects with simple level structures as light sources is that various types of photon correlations, such as the Brown-Twiss effect^{10,11}, Fresnel biases in superfluorescent fluctuations¹² and cascade effects¹³, are suppressed. In addition, if the fluorescence is sufficiently intense, the photoelectron fluctuations in the detectors can be treated classically; in these circumstances the instrumental jitter in the length of the telegraph pulses $\{t_i\}$ should be negligible⁸. However, random drifts in the detector gains, reference voltages and laser intensities, and interactions between the stored objects and the trapping and cooling fields, are unavoidable. Experiments are needed to determine whether the net effect of these interactions is to introduce additional elements of order or disorder.

In either case the search for correlations can be facilitated by starting with situations in which the fluorescence telegraph signals contain well-defined messages. An experimental precedent is the modulation of radioactive decay by pressure variations. One instance is the decay of the isomer ^{99m}Tc : this transition is inhibited by large changes of angular momentum and an energy release of only 2 keV. Accordingly, the decay proceeds mainly by internal conversion and the rate is very sensitive to the overlap of the initial and final electronic wave functions. This overlap can be altered by pressure variations, so that, for example, the $\sim 2.16 \times 10^4$ s half-life of ^{99m}Tc can be shortened by ~ 4 s if the ambient pressure is increased to ~ 0.1 Mbar¹⁴. Clearly, if the pressure were regularly cycled on a timescale shorter than 6 h, the existence of such periodic variations could be inferred from a stochastic analysis of the decay sequences. Similarly, the fluorescence signals from stored ions can be modulated by varying the intensities of the double-resonance lasers or by cyclically altering the trapping fields. As experiments based on the shelved electron scheme have already been carried out with 1–3 trapped $^{24}\text{Mg}^+$ ions stored within optical coherence lengths, additional information on correlations and field gradients could be extracted from the telegraph signals^{1,11,15}.

At the core of all these technical variations there remains the basic quantum-mechanical prediction that the decay time of the W-state is a random variable with an exponential distribution; this can be demonstrated rigorously using only the very weak assumptions¹⁶. In the specific case of the fluorescence telegraph this exponential behaviour is not affected by quantum measurement problems such as the 'watched-pot' or Turing paradox and observational asymmetries between prediction and retrodiction^{17,18}. The only possible source of intrinsic correlations in double-resonance measurements is a long-term quasi-periodicity that might affect the population of the S-state: however, independent experimental evidence on this point is still lacking¹⁹.

The fact that single atoms with minimal interactions with the environment can, in principle, generate arbitrarily long strings of random numbers is another remarkable aspect of quantum

mechanics. According to algorithmic complexity theory it is precisely the generation of random numbers that requires the maximum amount of complexity. In particular, n bits of information are required to specify the construction of n random binary numbers^{5,20}. The inherent indeterminacy of quantum jumps therefore not only constrains the ultimate precision of measurements but also implies that even the simplest physical systems are equivalent to arbitrarily large reservoirs of information.

The search for order in the fluorescence telegraph signals also has a bearing on the role of random variables in quantum mechanics. In the standard axiomatic formulation of probability theory, a random variable is defined as a measurable function on a probability space—where the probability space is essentially a set endowed with a normalized measure. The axiomatic development is deliberately silent concerning any requirements that the measurable functions be non-determinate or that the elements of the probability space correspond to inherently unpredictable or erratic events. Indeed, the axiomatic structure has "... applications in fields of science that have no relation to the concepts of random event and of probability in the precise meaning of these words"²¹. This basic lack of distinction between random and pseudo-random processes has several practical consequences for the cryptanalysis of the telegraph signals: (1) Inherently random events can sometimes emulate ordered patterns: for example, there is a small but positive probability that any correlation or apparent 'message' in the telegraph signals may actually be an artefact of chance events; this is the essence of the 'monkeys typing Hamlet' situation²². (2) A more serious concern is that deterministic processes can mimic random events. There is an extensive hierarchy of deterministic functions—including mixing transformations and K-systems—that can simulate the stochastic behaviour of random processes²³. These functions have recently come to popular notice in connection with studies of 'chaos'. Furthermore, the probability distributions implied by the Schrödinger wave functions of many systems are the same as the distributions which are associated with certain mixing transformations²⁴.

The nominally random decay sequences $\{e^{-\tau_i}\}$ of the fluorescence telegraph can also be simulated by the iteration of mixing transformations. For example, the functions

$$f(\tau) = -\ln(|2e^{-\tau} - 1|) \quad (2a)$$

$$g(\tau) = -\ln(|3e^{-\tau} - 1| - 1) \quad (2b)$$

where $\tau > 0$, are the beginning of a countably infinite sequence of mixing transformations, each of which simulates an exponentially distributed random variable. This means that if we begin with any arbitrarily chosen value of τ , say $\tau = 3.15$, and compute the successive iterates, that is, $f(3.15) \approx 0.0896$, $f[f(3.15)] = f^2(3.15) \approx 0.1880$ and so on, then term-by-term the sequence $\{f^i(3.15)\}$ appears to vary erratically, but a histogram would show that the values are in fact distributed with exponential weight $e^{-\tau}$. This behaviour is illustrated in Table 1. The fluctuations of the deterministic sequences $\{f^i(\tau)\}$ and $\{g^i(\tau)\}$ also satisfy the statistical criterion given by equation (1):

$$\langle |f^i(\tau) - f^j(\tau)| \rangle_{i,j} \approx 1 \approx \langle |g^i(\tau) - g^j(\tau)| \rangle_{i,j} \quad (3)$$

A random sequence would still pass this test even if only successive values were chosen

$$\langle |\tau_{i+1} - \tau_i| \rangle_i = 1 \quad (4a)$$

but the deterministic sequences have correlations that yield slightly different averages ($0 \leq i \leq 10^5$):

$$\langle |f^{i+1}(3.15) - f^i(3.15)| \rangle_i \approx 1.09; \quad \langle |g^{i+1}(3.15) - g^i(3.15)| \rangle_i \approx 0.92 \quad (4b)$$

Needless to say, more complicated simulations require more powerful cryptanalytical attacks to detect the underlying patterns of order.

Finally we consider the highly speculative question of whether there might be a physical basis for interpreting the randomness of quantum-mechanical variables in terms of the mixing gener-

Table 1 Simulation of exponential decay by a mixing transformation [equation (2b)]

τ	$g(\tau)$	$g^2(\tau)$	$g^3(\tau)$	$g^4(\tau)$	$g^5(\tau)$	...
3.15	2.0514	0.9528	0.1708	0.6368	0.8841	...
τ	0.05	0.15	0.25	0.35	0.45	... 2.25
$e^{-\tau}$	0.951	0.861	0.779	0.705	0.638	... 0.105
$\{g^i(3.15)\}^*$	0.959	0.862	0.780	0.698	0.637	... 0.100

* Histogram derived from 10^5 iterations; bin size $\Delta\tau = 0.1$; $0 \leq i \leq 10^5$.

ated by the deterministic iteration of appropriate functions. In the specific case of the fluorescence telegraph, there are two suggestive possibilities: (1) Suppose first we assume that the trapped ions that generate these signals have access to only a finite number of physical states. In particular, if we identify these states with cells in phase space—each of size $\hbar^3$ —it is possible to estimate their number N . For example, if a trapped ion is localized in a sphere of radius $2,000 \text{ \AA}$, and the electron transitions involve momenta bounded by 0.5 MeV c^{-1} , it is easy to check that the maximum number of accessible phase-space cells is $N \sim 10^{17}$. (2) Suppose further that the stored ion is sufficiently isolated from its environment so that it has access to only a finite amount of information²⁵. Physically this means that the net effect of the ion's interaction with the trapping fields, laser illumination and detectors is to induce a pseudo-random walk among the 10^{17} available phase-space cells. Under these conditions the telegraph sequences $\{e^{-\tau_i}\}$ will be deterministic simulations of a discrete random variable uniformly distributed in the interval $(0, 1)$. It can then be shown that after roughly $i \sim \sqrt{\frac{1}{2}\pi N} \sim 4 \times 10^8$ electron shelvings—or cycles of decay and re-set—each of the sequences $\{e^{-\tau_i}\}$ will merge into a terminal loop with about $\sqrt{\frac{1}{2}\pi N}$ elements. Moreover, no matter which of the 10^{17} possible states happens to initiate a telegraph signal, there is an $80 \pm 1\%$ probability that the corresponding pseudo-random sequences $\{e^{-\tau_i}\}$ will merge into the same terminal loop⁶. The practical implication of these results is that if sufficiently long strings of signals are analysed, the existence of 'messages' or terminal loops will eventually be detected. Such messages are not contained in the usual formulation of quantum theory. Since the basic cryptanalytical signature of this type of deterministic behaviour is the repetition of patterns, the relative precision of the individual observations—such as the duration of the telegraph pulses t_i and T_i —does not of course have to be comparable to one part in 10^{17} or 10^8 !

We thank P. Hammerling for helpful discussions and G. Hockney for undertaking the computer simulations.

Note added in proof: A double resonance experiment with stored $^{198}\text{Hg}^+$ ions is currently being assembled at National Bureau of Standards (Boulder, Colorado). Its characteristics are: (1) lifetime of strong transition $\sim 10^{-9} \text{ s}$; (2) Rabi flopping frequency $\geq 10^7 \text{ Hz}$; (3) photon collection efficiency $\sim 10^{-3}$; (4) number of photons detected per telegraph pulse $\sim 10^3$. Since this experiment is intended for precise frequency measurement the lifetime of the weak transition is $\sim 0.1 \text{ s}$! A faster weak transition would facilitate the search for patterns (D. Wineland, private communication).

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Dual emission-line regions in the Seyfert galaxy NGC 5929

William C. Keel

Kitt Peak National Observatory, National Optical Astronomy Observatories, PO Box 26732, Tucson, Arizona 85726-6732, USA

Several theoretical and empirical studies suggest that multiple distinct emission regions exist in the nuclei of some active galaxies. Detailed study^{1,2} of nearby objects such as M51 shows that the most important radiation of nonstellar origin can originate hundreds of parsecs from the nucleus (as defined by the stellar potential well), while binary compact objects are expected in some cluster-collapse schemes³ or in interaction-fuelled models for active nuclei^{4,5}. Here I show that the type 2 Seyfert nucleus of NGC5929 contains two emission regions situated symmetrically about the centre of the galaxy. Slit spectroscopy shows them to be spatially as well as kinematically distinct, each with a linewidth of $\sim 200 \text{ km s}^{-1}$ and ionization levels normally associated with Seyfert nuclei. Their location in the galaxy's rotation curve suggests that they move with the galaxy's disk gas; and they probably result from energy transport from a central object of low photon luminosity but high energy output. This system furnishes a very clear example of the presence of phenomena related to the presence of a central engine, but located at a significant distance from the ultimate source of energy. The existence of such objects suggests that the usual radiatively powered, nearly symmetric models for active nuclei may be misleading in many objects.

Previous data on the Sab galaxy NGC5929 indicated that two nuclei, or discrete emission regions, might be present. It was first identified as a type 2 Seyfert by the CfA group⁶. Intermediate-resolution aperture spectra (ref. 7 and J. Stauffer, personal communication) showed the emission-line profiles to be double-peaked. A Very Large Array (VLA) map at 6 cm by Ulvestad and Wilson⁸ showed emission dominated by two unresolved sources 1.3 arc s apart in position angle 60° , and some evidence for a weak source between them.

To confirm an association between the spatially distinct radio sources and kinematically distinct emission-line regions, a high-resolution spectrum has been obtained along the line defined by the radio sources. A Texas Instruments 800×800 charge-coupled device (CCD) was used with the Ritchey-Chrétien spectrograph on the 4-m Mayall telescope, covering the wavelength range $6,300\text{--}6,750 \text{ \AA}$ at a resolution (FWHM) of $2.0 \text{ \AA}$, set by the slit width of 1.0 arc s . A 1-h exposure was obtained, resulting in peak levels of 700 counts (3,000 detected photons) per pixel ($0.64 \text{ \AA}$ by 0.31 arc s) in the emission lines. The spectrum clearly shows two emission regions near the nucleus, kinematically and spatially separate (Fig. 1). The $[\text{O I}] \lambda 6,300, 6,363$ lines appear in both; this fact, the similar velocity widths ($\sim 200 \text{ km s}^{-1}$ FWHM), and high $[\text{O III}]/\text{H}\beta$ in both components as found from previous data⁷, indicate that each of these regions has properties normally associated with active nuclei. The ionization levels are clearly most like type 2 Seyferts or narrow-line radio galaxies, despite the rather small linewidths. In this case, straightforward classification as a type 2 Seyfert may have obscured recognition of the unusual character of the

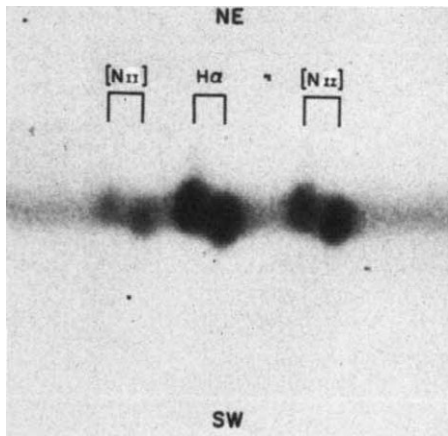


Fig. 1 The spectrum of NGC5929 in the region of $H\alpha$ and $[N II]$ $\lambda\lambda 6,548, 6,583$. The two emission regions are separated by 291 km s^{-1} and 1.3 arc s ; most of the spread along the slit is internal to the spectrograph camera. Additional weak emission between the two main peaks may be seen at $H\alpha$.

object, but the integrated line ratios and linewidths are certainly those associated with Seyfert nuclei.

Quantitative measures of the individual spectra have been performed by multiple-component gaussian fits to blended lines in each row of the spectrum, accounting for contamination of each spectrum by the other. The line ratios, relative strengths in $H\alpha$, and linewidths corrected for the instrumental profile are given in Table 1. Subtraction of the modelled components from the data provides some evidence of a weak, broad $H\alpha$ line at the centre of the galaxy; part of this emission may be seen between the $H\alpha$ peaks in Fig. 1. A comparison of the spectra of the two regions, obtained by taking the rows of the spectrum at the outer edges of each emission-line system to minimize scattered light from the other, is shown in Fig. 2.

Additional imaging data were obtained (in collaboration with R. Kennicutt) using the same CCD at the KPNO 2.1-m telescope. Exposures through filters with $75\text{-}\text{\AA}$ passbands centred on and off $H\alpha$ were obtained. Figure 3 shows the galaxy's morphology, its interacting companion NGC5930, and an enlarged portion of the $H\alpha + [N II]$ difference image. The emission-line structure is consistent with two unresolved centres of emission blended by seeing effects, with a separation close to that of the radio sources but a significantly different position angle ($\sim 85^\circ$). The structure is not consistent with a ring of emission, which might give similar spectroscopic results but a quite different physical picture.

From these data, several characteristics of the system are clearly established. Two dominant emission-line regions exist, each with a luminosity⁷ and ionization level sufficient for classification as a Seyfert 2 nucleus. They are separated by 1.3 arc s (projecting to 190 pc at an assumed distance of 34 Mpc derived by assuming $H_0 = 80 \text{ km s}^{-1} \text{ Mpc}^{-1}$), and have a radial-velocity difference of $291 \pm 11 \text{ km s}^{-1}$. The optical emission is nearly coincident with the pointlike radio sources. Finally, neither emission region coincides with the galaxy's dynamical centre; they fall instead along the rotation curve defined by disk $H II$ regions (easily traced in these data but not well seen in the low-contrast representation of Fig. 1), on either side of the nuclear velocity.

At least two plausible pictures are available to account for this configuration. Actual double nuclei, powered by separate compact objects, are occasionally expected when activity has been induced by a merger or cascading collapse of a stellar core to form compact objects. Gaskell⁹ has presented evidence for offset broad-line profiles in quasars, interpreted as evidence for merger-triggered activity, with the broad-line regions representing the cores of one of the original galaxies. In such a scheme,

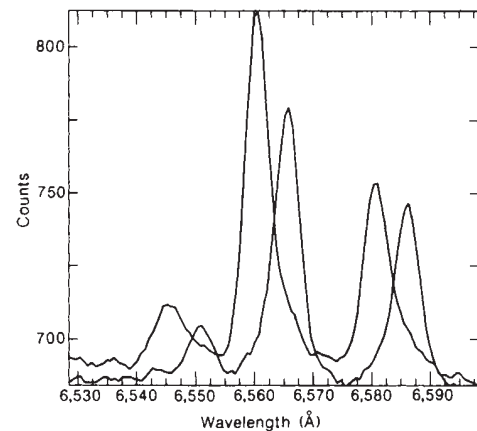


Fig. 2 Cuts through the data of Fig. 1 at the top and bottom of the emission regions, showing the spectral properties of each free from contamination by scattered light from the other. The signal-to-noise ratio is consequently lower than that obtained at the peaks, where the images of the two regions overlap along the slit due to atmospheric and instrumental spread. The wavelength scale is in the frame of the dynamical centre of NGC5929, which has an observed radial velocity of $2,516 \text{ km s}^{-1}$.

one compact object would be expected at or very close to the centre of the dominant galaxy. In most cases, a large mass ratio for the compact objects would be expected, and probably reflected in the luminosities, simply because mergers of two galaxies with nearly equal masses of the central objects are unlikely, given the large range in level of nuclear activity seen at a given galaxy luminosity.

The picture is not likely to apply to NGC5929 for several reasons. The equal spacing of the emission regions about the dynamical centre of the galaxy implies that neither represents a central object which has long existed in this galaxy. The separation of the two emission regions is too large for a binary formed by core collapse, and probably too large to represent remnant cores in a merger which has had time to produce a normal disk. The presence of a central source of radio (and perhaps broad $H\alpha$) emission would not be naturally explained. Additional circumstantial evidence against a recent merger is the fact that NGC5929 is now seen interacting with NGC5930, so that a recent merger would require that the system now be seen at a very special time.

An alternative explanation is suggested by objects showing evidence for substantial unseen energy transport between the central engine and emission regions at distances from hundreds of parsecs (as in M51^{1,2}) to kiloparsecs (radio galaxies¹⁰). The detected emission regions in these cases seem to be powered by the nucleus, but through a beamed (or 'fanned') mechanism analogous (and perhaps identical) to jets¹¹. NGC5929 may be considered within this framework as an unusually clear example of traditional active regions powered by the nucleus while lying at some distance from it. The weak central source seen at 6 cm and suspected at $H\alpha$ would then be radiation from the immediate vicinity of the central engine, while the total output of the nuclear region is dominated by twin emission regions which convert nuclear (radiation or particle) beams into line radiation (with some continuum seen at least in the radio).

The offset between radio and emission-line peaks, in this

Table 1 Emission-line systems in the nucleus of NGC5929

Line ratio	NE	SW
$[N II] \lambda 6,583/H\alpha$	0.51	0.67
$[S II] \lambda\lambda 6,717, 6,731/H\alpha$	0.59	0.81
$[O I] \lambda 6,300/H\alpha$	0.18	0.31
$[S II] \lambda 6,717/\lambda 6,731$	1.14	1.11
Relative $H\alpha$ flux	0.48	0.52
FWHM (km s^{-1})	174	192

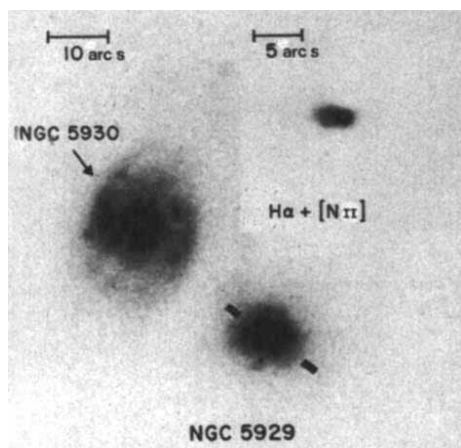


Fig. 3 NGC5,929/5,930 as observed in a 75-Å passband including H α . The orientation and width of the spectrograph slit used to obtain the data of Figs 1 and 2 are shown by the black lines. Inset, an enlargement of the nucleus of NGC5929 after subtraction of a continuum image, yielding a pure emission-line image. The bright portion of this image is consistent with two sources unresolved at the 1 arc s level, separated by ~ 1.2 arc s.

picture, presumably indicates that the radio peaks mark the sites of most intense ionization by the nuclear jets (or other, still collimated, forms of energy transport). The extent of optical emission is governed as much by the availability of gas as by the amount of energy available to power the emission. As there is no clear mechanism by which the directions of energy output from the nucleus should be coupled to the disk rotation 100 pc away, a 'tail' of excited material can develop as new matter is brought into the beams. The emitting clouds will become misaligned by a measurable amount with their starting points in $\sim 10^5$ yr; if the gas at $n_0 \approx 10^3 \text{ cm}^{-3}$ survives longer than this after first being ionized by the nucleus, a characteristic S-shaped configuration (like that frequently attributed to precessing jets) will be seen in the emission lines; when not resolved, this would lead to an observed centroid position offset from the points of current energy input.

If the nucleus supplies energy to the emitting region primarily in particles, they may have a role in producing the observed densities of gas through compression of the existing interstellar medium, and may also contribute to the internal velocity dispersion of the clouds. If the particle beams take the form of discrete plasmons, which would be seen directly as the 6-cm sources, the model elaborated by Pedlar *et al.*¹² to account for the densities and velocity widths in Seyfert 2 nuclei as a class might apply. In this model, the expanding plasma clouds compress the surrounding interstellar medium during thermal expansion; the medium cools after the expansion slows below $\sim 400 \text{ km s}^{-1}$ to produce the emission-line region. Some analogous process, whether discrete or continuous ejection takes place, is quite likely to be active in NGC5929.

The properties of this nucleus suggest interesting implications for other active nuclei. Most narrow-line regions remain spatially unresolved, especially in distant, high-luminosity objects. If excitation by beams, in addition to direct photoionization from the nuclei, is important, elongated or double emission regions may be quite common, and are to be expected in some abundance with the resolution attainable with the Space Telescope. Also, note that the genuine compact object in NGC5929, identified as the 6-cm central component and perhaps a broad H α source, is very inconspicuous, and would be difficult to detect in the absence of a surrounding gaseous disk to produce the prominent narrow-line regions. Linking statistics of active nuclei with those of central compact objects will clearly require detailed knowledge of the gaseous environments of the nuclei involved.

These data indicate that nuclear activity in galaxies can take forms significantly different from the picture developed for most

Seyfert galaxies, in which a central source of ionizing photons powers a more or less spherical ensemble of surrounding gas clouds, which then produce the emission-line spectrum. In at least some cases, the emission-line regions can be far from the energy source and dynamical centre of the galaxy, and a large part of the energy supply for these regions is asymmetrically generated by the central object. Note that if only one of the emission regions were present in NGC5929, its peculiarity would not have been detected without considerably more detailed investigation. In at least one other galaxy, NGC2110, the emission-line intensity and width peak at a projected distance of 220 pc from the kinematic centre¹³. In view of the results for NGC5929, it may be argued that such objects as NGC2110 contain 'one-sided' counterparts of the double emission-line region, and that more active nuclei may exhibit such properties when observed at high spatial resolution.

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An early-medieval account on the red colour of Sirius and its astrophysical implications

Wolfhard Schlosser* & Werner Bergmann†

Department of * Physics and Astronomy and † History, Ruhr-University Bochum, Postfach 10 21 48, D-4630 Bochum 1, FRG

An unresolved problem regarding ancient astronomical records is that of the star 'Red Sirius'. While Sirius today shines white with a blueish hue quite in agreement with its spectral type A1V, many Greek/Roman and Babylonian sources (although still disputed) definitely assign a red colour to this star. We now present new and apparently independent information about Red Sirius from an early-medieval manuscript. This manuscript is of Lombardic origin (8th century) and contains the otherwise lost '*De cursu stellarum ratio*' by Gregory of Tours (about AD 538-593). It is preserved in the library of Bamberg¹. Red stars in ancient records are those with colour index $B - V = 1.0$ or greater. Assuming an unchanged Sirius A, this lower limit for the combined colour of Sirius A and B allows the computation of the region of pre-white dwarf Sirius B in the Hertzsprung-Russell diagram or colour-magnitude diagram (Figs 1, 2). Sirius B lies on the giant branch, which fits well with our observational and theoretical framework of stellar evolution. However, the timescale of transformation of a red giant to a white dwarf is surprisingly short.

In antiquity, Sirius was considered generally as a red star. A well-known case is Ptolemy's reference to Sirius as 'reddish' in his *Almagest*. Other classical authors, including Cicero, Horace and Seneca, concur with this opinion. Less well known is the

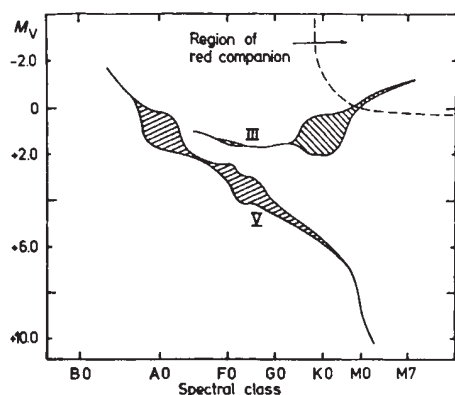


Fig. 1 Observed Hertzsprung-Russell diagram for luminosity classes III and V (after ref. 7). The variable width of each sequence indicates its varying population. The giant branch (III) runs into the region of the hypothetical red companion of Sirius A. Conversion of colour into spectral class is after ref. 9.

fact that the Romans sacrificed red-coated dogs to Sirius at the time of its heliacal rise².

The red colour of Sirius in antiquity is further substantiated by many Babylonian cuneiform texts. In his *Planetarium Babylonium*, Gössmann³ quotes many astronomical and astrological qualities attributed to Sirius. (We follow Gössmann's translation, although not all scholars agree on the assignation of Babylonian terminology to its modern equivalent (see, for example, ref. 4).) If a colour is given, it is always red. Far-Eastern texts do not give any hint of a colour change for Sirius.

Since such a marked change of colour within historical times seems hard to accept, two alternative explanations were considered. The first one attributes the colour change to atmospheric reddening. Within 3° of elevation above horizon, Sirius indeed assumes or even exceeds the red colour of Betelgeuze. This, however, is restricted to 15 min after rising or before setting and is by no means representative of the 11-h diurnal arc of Sirius. The second argument is based on colour scintillation, which randomly changes the colour of bright stars close to the horizon. Here, simple observations of this phenomenon confirm intuitive physical insight: a white star produces as many red flashes as green or blue ones.

One should also remember that Sirius is not the brightest body in the sky. Leaving out the Sun and Moon, Sirius ranks fourth after Venus, Jupiter and Mercury, where no such controversy exists.

We have now found new information about the Red Sirius while analysing Gregory of Tours' *De cursu stellarum ratio*. The purpose of this work was to furnish monasteries with clear instructions on when to conduct nocturnal divine services, and for this, the rise-times of certain constellations for different months are listed. Since Gregory of Tours lived about one century before the rise of Islam, no Arabic star names appear in the manuscript, but none of the well-known classical names still in use today is used either, so this source reflects an astronomical tradition independent of classical or Babylonian skylore.

In this merovingian text, Sirius is easily identified by its rise-times and times of visibility. It is called 'stella splendida', the only case in the manuscript where this attribute of highest luminosity is given. Its proper name is 'Rubeola' or 'Robeola', meaning 'red' or 'rusty'.

This new and seemingly independent evidence for the red colour of Sirius in the past suggests that there might indeed have been an astrophysical transformation of the Sirius A/B system during the past two millennia. From an astrophysical viewpoint, the white dwarf Sirius B is the better candidate for such a change, since white dwarfs are thought to be the result of a collapsed red giant.

The total brightness of an unresolved binary system is obtained simply by adding up the intensities of each component.

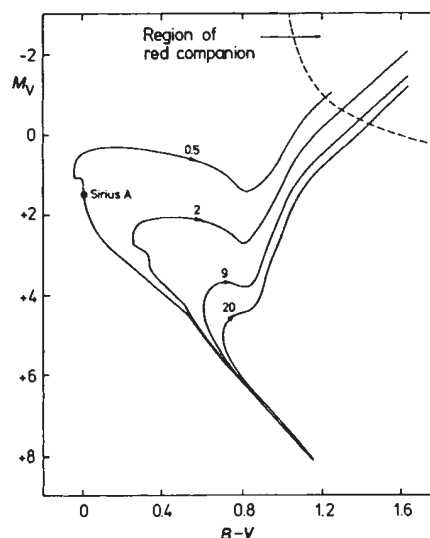


Fig. 2 Computed evolution of stars of the same age but different masses ($0.6-3 M_{\odot}$) from main sequence to the region of red giants (arrows). The direction of evolutionary tracks coincides well with predicted pre-white dwarf Sirius B colours and magnitudes. (Simplified after ref. 8 for 30% helium content and 1% heavier elements.) The numbers refer to ages in $\text{yr} \times 10^9$. Present-day Sirius A lies well on the main sequence, a further hint of its stability during the past two millennia.

Basically, the same is true for the colours. Thus, a colour-magnitude relation for Sirius B in the past may be computed by assuming an unchanged Sirius A and guessing the colour of the combined light. In antiquity, the following stars were considered to be red (Sirius excluded); Betelgeuse ($B-V=1.9$), Antares ($B-V=1.8$), Aldebaran ($B-V=1.5$), Mars ($B-V=1.4$), Arcturus ($B-V=1.2$) and Pollux ($B-V=1.0$). (All colours $B-V$ are from refs 5, 6.) Since Ptolemy's *Almagest* assigns no specific colour to α Centauri ($B-V=0.7$), a lower limit of $B-V=1.0$ is probably a good approximation for stars called 'red' in antiquity. Using this lower limit, a straightforward computation confines Sirius B to the region specified in Figs 1 and 2. It agrees well with the giant branch in the observed Hertzsprung-Russell diagram⁷ and the evolutionary tracks of a broad variety of stellar masses according to theoretical astrophysics⁸.

If Sirius was redder in antiquity, it must also have been brighter. Taking absolute visual magnitude, $M_V=+1.43$ for Sirius A and assuming $M_V=-1.0$ for Sirius B before change (Figs 1, 2), one obtains $M_V=-1.11$ for the combined light. With a distance modulus $m-M=-2.89$ this gives a total magnitude of -4.0 , which is about that of Venus. Like Venus, Sirius should have been visible in daylight under favourable conditions. Indeed, several Babylonian sources mention the daytime visibility of Sirius³.

Thus, Sirius B might well have changed from a red giant to the white dwarf as it appears today. However, the rapidity and smoothness of this transformation are quite unexpected, and its timescale is surprisingly short. Furthermore, no traces of catastrophic effects connected with such an event have been found. The only indication that something has happened is the somewhat higher metallicity of Sirius A, believed to have resulted from contamination by the giant's blown-off shell.

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Possible nightside source dominance in nonthermal radio emissions from Uranus

S. A. Curtis

Planetary Magnetospheres Branch, NASA/Goddard Space Flight Center, Greenbelt, Maryland 20771, USA

Desch and Kaiser¹ have formulated a radiometric Bode's law from which they have attempted to estimate the low frequency, non-thermal radio power of Uranus' magnetosphere likely to be measured by the Voyager 2 spacecraft as a function of the planet's magnetic moment and the solar wind power input. They have in essence calculated what are possible necessary conditions for the generation of radio emission from Uranus. A potential problem arising from this calculation is that although their model for uranian radio emission may represent necessary conditions, they are not sufficient. I show here that, if Uranus possesses a magnetosphere, it is more likely that the radio emission is from the nightside as opposed to the dayside as assumed by Desch and Kaiser. A nightside source for the radio emissions would radically alter the predicted time for first observations of the emissions.

If a spacecraft were to approach the Earth, whose emission source is on the near-midnight high latitude nightside, from the inside its orbit, or if it were to approach Saturn, whose emission source is on the near-noon, high latitude dayside from outside its orbit, in both cases emissions would not be detected until very near encounter. Thus, although the power levels at both planets exhibit a degree of scalability, they have very different emission regions and directions that would affect any attempted predictions based on power levels alone. Earlier related work by Hill and Dessler² whose main point was to explain the ultraviolet auroral emissions using a somewhat different magnetospheric structure, qualified their predictions concerning radio emissions. In particular, they noted the uncertainty in the interpretation of the radio emissions owing to differences in beaming geometries between the planets.

One does indeed require an overall power input to a planetary magnetosphere to provide the free energy to drive planetary radio emissions. However, this energy still needs to be put in a usable form, generally field-aligned fluxes of weakly relativistic keV electrons in a region where the electron plasma frequency to electron gyrofrequency ratio, $\omega_{pe}/\omega_{ce} < 1$ (refs 3-5). More specifically, Benson⁶, using ISIS-1 satellite observations of the Earth's auroral kilometric radiation (AKR) has shown that $\omega_{pe}/\omega_{ce} < 0.2$ for the generation of extraordinary (X) mode radiation. Benson also deduced generation of weaker ordinary (O) mode radiation is allowed for $\omega_{pe}/\omega_{ce} \leq 0.9$. Since $\omega_{pe}/\omega_{ce} = 3.21 \times 10^{-3} n_E^{1/2} B^{-1}$, where n_E is the electron density in cm^{-3} and B is the magnetic field in gauss, the ω_{pe}/ω_{ce} limits of Benson yield constraints on the generation of low frequency radio emission on Uranus.

A unique aspect of Uranus is compared to Earth, Jupiter and Saturn is it's near pole-on orientation towards the Sun and possible magnetic pole orientation in nearly the same direction. The possible existence of an intrinsic magnetic field has been revealed by the International Ultraviolet Explorer (IUE) spacecraft's⁷ apparent detection of uranian auroral emission that are consistent with a magnetosphere. The region of open field lines called the cusp that allows access to the ionosphere of Uranus by the shocked solar wind is thus located near the subsolar point of the planet as opposed to higher latitudes at Earth, Jupiter and Saturn. Magnetosheath plasma may thus have more direct access to the ionosphere via the cusp throat than at Earth. Given the collisional ionosphere at the base of the cusp and the cross-magnetic field transport required to move plasma outward through the cusp walls, we see that, unless a strong convective electric field is set up by solar wind/magnetosphere interaction, the cusp plasma will act as a local plasma source for the ionosphere at its base. We note that the magnitude of the cusp plasma source may be highly variable owing to solar wind dynamical evolution by entrainment and pressure waves as

discussed by Burlaga *et al.*⁸. Additionally, if these density variations are sufficiently great, the situation may become similar to the immersion of Saturn in the jovian tail⁹ and no substantial emission would be expected from Uranus in the low density solar wind troughs.

For X-mode generation we have the constraint $n_E < 3.9 \times 10^3 B^2$, and similarly for O-mode, $n_E < 7.9 \times 10^4 B^2$. Hence for $B \approx 0.1$ gauss (Earth-like), $n_E \geq 39 \text{ cm}^{-3}$ will suppress X-mode and $n_E \geq 790 \text{ cm}^{-3}$ will suppress O-mode also. These are very low densities for a dayside topside ionosphere with the additional cusp plasma source and unless the magnetic field is strong (≥ 1 gauss), it appears that both emission modes will be suppressed.

An additional limitation posed by high cusp electron densities is the difficulty in maintaining field-aligned electric fields needed to produce the energetic keV electron fluxes. The high densities will tend to short them out and preclude wave trapping acceleration mechanisms as have been proposed for producing field-aligned keV electron fluxes by Hasegawa¹⁰ which are needed for the Earth's AKR.

Having shown that it is plausible that a dayside source of uranian radio emission does not exist, I next consider possible nightside generation. In this case the cusp densities should be much reduced owing both to the lack of photoionization as well as the inhibition of transport of dayside ionospheric plasma to the nightside across magnetic field lines. Thus if $B \sim 0.1$ gauss Earth-like auroral zone type densities should be possible with $\omega_{pe}/\omega_{ce} < 1$ if $n_E < 10\text{--}100 \text{ cm}^{-3}$. Thus strong emission will occur provided the keV electron fluxes can be produced in sufficient quantity. To explore this, I examine two limiting cases of the uranian magnetosphere. These two cases represent (1) the limits of high mass loading due to pickup plasma by the magnetosphere from the planet's satellites resulting in a rotationally driven, convection-dominated magnetosphere¹¹ like Jupiter and Saturn or (2) at the opposite extreme, no mass-loading and a Faraday disk dynamo magnetosphere¹². In the first case the emissions may be expected to be dominated by plasmashet related MHD instability processes and electron acceleration by wave-trapping as they apparently are at Saturn¹³. In contrast, in the low mass-loading limit, one would expect the Kelvin-Helmholtz instability operating on the magnetopause boundary to generate field-aligned surface MHD waves. Through mode conversion¹⁰, these waves will trap and similarly accelerate magnetosheath electrons into the nightside cusp/magnetosphere boundary region. Since the waves will only be driven nightward, the electrons will be accelerated only in the direction of the nightside pole. This limiting case presents some difficulties if the IUE observations are correct, since only a nightside auroral and radio emission would be expected. In contrast, in the high mass-loading limit, the centrifugally drive plasmashet interchange instabilities can drive surface waves that can accelerate electrons into both dayside and nightside cusp regions. In this case, since $\omega_{pe}/\omega_{ce} > 1$ in the dayside cusp-magnetosphere boundary region, only aurora would be generated on the dayside. However, both aurora and radio emission can be generated on the nightside where $\omega_{pe}/\omega_{ce} < 1$ is likely. Hence, the dominant radio emission from Uranus is expected from the nightside cusp-magnetosphere boundary region, which should not be observed until uranian encounter. We also note that of the dayside aurora of Uranus are genuine, mass-loading of the magnetosphere by its satellites is probably an important process within the framework presented here. If, however, planetary rotation and magnetic merging combine to give a large enough helical twist to the tail field lines, the resulting Birkeland currents that flow into the dayside polar cap may give rise to a dayside polar aurora even in the low mass-loading limit¹².

In conclusion, it is not possible to exclude either the Desch and Kaiser, or the Hill and Dessler power scaling relations. Clearly, however, their predictive power is marginal in that all of these authors have assumed a dayside radio emission source on Uranus which may, according to the arguments presented here, be either very weak or non-existent.

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Predicted chemistry of the deep atmosphere of Uranus before the Voyager 2 encounter

Bruce Fegley Jr & Ronald G. Prinn

Department of Earth, Atmospheric, and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

The Voyager 2 spacecraft will encounter Uranus in January 1986, and will provide the first spacecraft observations of that planet. It carries an infrared interferometer spectrometer (IRIS) which has already detected a variety of infrared active gases in the atmospheres of Jupiter, Saturn and Titan¹⁻⁴. Some of the detected gases are not in chemical equilibrium but have sources in the deep atmosphere. IRIS observations of these non-equilibrium gases, in particular PH₃ (mixing ratio $X_{\text{PH}_3} \approx 6 \times 10^{-7}$) and GeH₄ ($X_{\text{GeH}_4} \approx 7 \times 10^{-10}$) on Jupiter¹ and PH₃ ($X_{\text{PH}_3} \approx 1 \times 10^{-6}$) on Saturn³, can be used to deduce the strength of convective mixing in the deep atmospheres of Jupiter and Saturn^{5,6}. Similar deductions could be made about convective mixing rates in the deep atmosphere of Uranus if models of the equilibrium chemistry and thermochemical kinetics were available to help interpret IRIS observations of the visible Uranus atmosphere. We describe here the results of comprehensive thermochemical equilibrium and chemical kinetic calculations for Uranus. We predict that the most abundant non-equilibrium trace gas derived from the deep atmosphere of Uranus is N₂; other important non-equilibrium species include HCl, HF, GeH₄, C₂H₆, PH₃, H₂Se, CH₃SH, CO, CH₃NH₂, CH₃OH, and CO₂. Some of these species are detectable potentially by the Voyager instruments.

We assume that Uranus, like Jupiter and Saturn, has a deep convective atmosphere. Use of this assumption also leads to reasonable interior structure models⁷, but this is still uncertain because of the apparent lack of a large internal heat source on Uranus⁸. The present results refer to an arbitrary but plausible compositional model in which H₂ and the noble gases (He, Ne, Ar, Kr, Xe) are present in solar abundance⁹, H₂O, NH₃, H₂S and many 'heavy' elements (atomic number ≥ 3) are enriched 100 times relative to solar abundance, and CH₄ is enriched ≈ 10 times. This model, which is based on the low-temperature equilibrium condensation sequence outlined by Lewis¹⁰ and Prinn and Fegley¹¹, is one of several that we are investigating. It is consistent with deductions from visible¹² and infrared¹³ spectroscopy and interior structure models⁷ that Uranus is enriched in 'heavy' elements relative to solar abundances.

We constructed an adiabatic temperature-pressure profile for our model Uranus atmosphere taking temperature $T = 74$ K at pressure $P = 1$ bar (ref. 13). Thermochemical equilibrium calculations were done for several hundred compounds of the elements H, He, O, C, N, S, Fe, Mg, Si, Na, Cl, K, F, P, Ge, Se, Ne, Ar, Kr, Xe, Ca, Al and Ti. The thermochemical kinetics of the important destruction reactions for selected gases were also studied. Homogeneous gas phase and heterogeneous Fe-catalysed destruction reactions for N₂ and CO were included in our model. The specific techniques used to construct our model and the thermodynamic and chemical kinetic data sources are described in detail elsewhere^{5,6,11,14-18}.

The calculated equilibrium mixing ratios of important gases as functions of temperature and pressure are illustrated in Fig. 1. The abundances of two major gases H₂O and NH₃ are appreciably reduced by the formation of dilute NH₃-H₂O solution clouds at 595 K. By the 275 K level the NH₃ abundance is less than the H₂S abundance and it is quantitatively removed from the atmosphere by precipitation of NH₄HS(s) as first suggested by Prinn and Lewis¹⁹. X_{NH_3} varies from about 10^{-9} at 200 K to 10^{-14} at 160 K, compatible with the observations of Gulkis *et al.*²⁰ that NH₃ is depleted in this region of the uranian atmosphere. Atreya and Romani²¹ and Stevenson²² also noted that formation of NH₃-H₂O solution clouds would consume large amounts of NH₃. The excess H₂S may condense slightly higher in the atmosphere at 166 K but is probably photolysed to S₈(s) on a short timescale, while CH₄(s) condenses at 77 K. The equilibrium abundances of other gases such as HCl, HF, H₂Se, GeH₄ and P₄O₆ are reduced significantly by precipitation reactions lower in the atmosphere (see Fig. 1).

Larger enhancements of H₂O will yield solution cloud condensation at higher temperatures (and deeper levels) of the

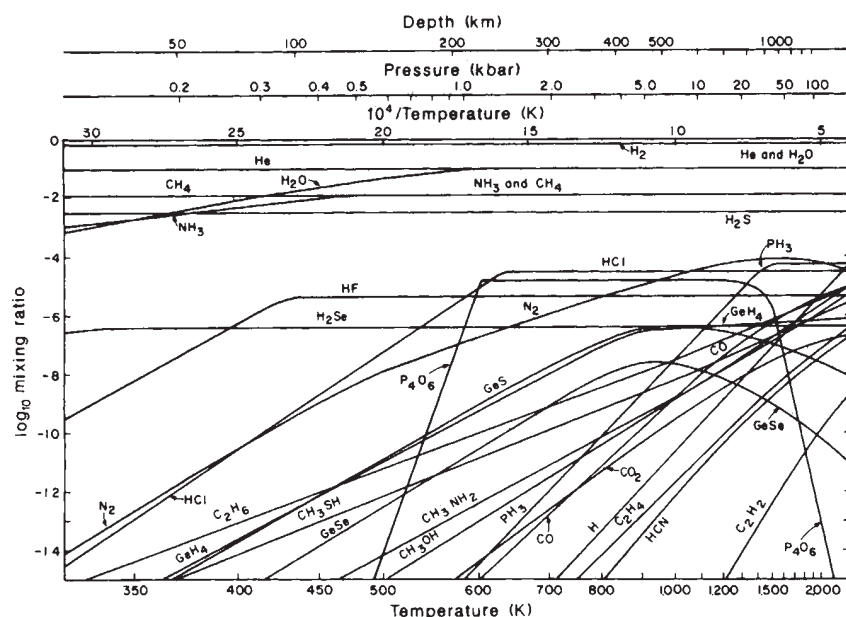


Fig. 1 Equilibrium abundances of important gases along the model adiabat in the atmosphere of Uranus. The horizontal scales indicate temperature, depth below the 300 K level and pressure along the adiabat. The constant abundances of the noble gases ($X_{\text{Ne}} = 1.5 \times 10^{-4}$, $X_{\text{Ar}} = 6.1 \times 10^{-6}$, $X_{\text{Kr}} = 2.4 \times 10^{-9}$ and $X_{\text{Xe}} = 3.4 \times 10^{-10}$) are not plotted. Precipitation of NH₄Cl(s), NH₄F(s), NH₄H₂PO₄(s), Ge(s) and GeSe(s) at 629 K, 430 K, 602 K, 868 K and 339 K, respectively, depletes HCl(g), HF(g), P₄O₆(g), Ge gases and Se gases. Solution of H₂S(g) and HF(g) in the ammonia-water clouds is a minor effect. All compounds of Fe, Mg, Si, Na, K, Ca, Al, Ti are condensed phases over the (P, T) range considered.

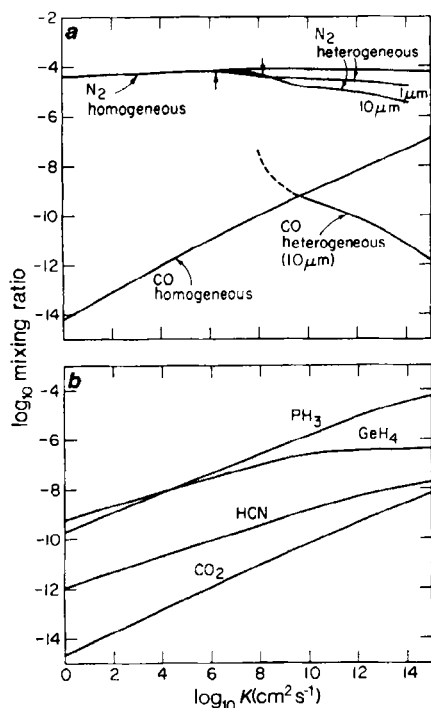


Fig. 2 *a*, Predicted N_2 and CO mixing ratios in the observable Uranian atmosphere as a function of the vertical eddy diffusion coefficient K . Homogeneous gas-phase and heterogeneous iron-catalysed reactions are considered. Curves for catalysis on iron cloud particles with radii of $10\ \mu\text{m}$ (CO) and $1\ \mu\text{m}$ and $10\ \mu\text{m}$ (N_2) are shown. The dotted line on the CO heterogeneous curve indicates the predicted trend in the absence of homogeneous gas-phase reactions. Actual CO mixing ratios for $K < 5 \times 10^9\ \text{cm}^2\ \text{s}^{-1}$ will follow the homogeneous curve. *b*, As *a* but for PH_3 , GeH_4 , HCN and CO_2 . Homogeneous gas-phase reactions only are considered.

Uranian atmosphere until the H_2O critical point of 647 K is reached. Because the initial clouds are very dilute NH_3 - H_2O solutions which have critical points slightly lower than that of pure water²³, the 647 K level is the upper temperature limit to a NH_3 - H_2O solution cloud base on Uranus. Latent heat effects due to solution condensation will also decrease as the cloud-base temperature approaches the critical point for the first formed (dilute NH_3 - H_2O) solution^{24,25}.

The observations of PH_3 and GeH_4 on Jupiter¹ and of PH_3 on Saturn³ at concentrations orders of magnitude greater than their equilibrium abundances in the cool visible regions of the jovian and saturnian atmospheres have been interpreted in terms of rapid convective mixing from the hot deep atmospheres where these species are more abundant^{5,6,14}. We have applied a similar model to Uranus to predict concentrations of non-equilibrium gases such as N_2 , GeH_4 , PH_3 , CO, CO_2 , and HCN in the Uranian upper atmosphere as a function of the rates of the relevant homogeneous and heterogeneous destruction reactions and the (assumed) strength of convective mixing in the deep Uranian atmosphere. Iron-catalysed heterogeneous $N_2(g)$ and $CO(g)$ destruction reactions were considered using the model of Prinn and Olaguer¹⁷. This treatment gives firm lower limits to the N_2 and CO mixing ratios, because Fe(liquid) condenses at 6,000 K (2.4×10^7 bar) so Fe particles in the 1,000–2,000 K region are unlikely. The results of these calculations are illustrated in Fig. 2 where the predicted mixing ratios are plotted as a function of the vertical eddy diffusion coefficient K .

No observational estimates are available for the vertical eddy diffusion coefficient K in the deep Uranian atmosphere. However, various *a priori* estimates of K are possible. Assuming that free convection is a dominant mode for vertical heat transport in the deep Uranian atmosphere, we can estimate K from the relationship, $K \approx H(\phi/\rho\gamma)^{1/3}$ where H is the pressure scale

Table 1 Predicted non-equilibrium trace gas abundance in the observable atmosphere of Uranus for 100 times enriched compositional model ($K = 10^7$ – $10^8\ \text{cm}^2\ \text{s}^{-1}$)

Gas	Quench temperature (K)	Predicted mixing ratio
N_2	1,205–1,390	$(5.0\text{--}6.3) \times 10^{-5}$
PH_3	1,058–1,102	$(1.0\text{--}2.3) \times 10^{-7}$
GeH_4	775–810	$(5.0\text{--}10) \times 10^{-8}$
CO	846–885	$(3.2\text{--}10) \times 10^{-11}$
HCN	1,299–1,357	$(1.6\text{--}3.5) \times 10^{-10}$
CO_2	787–832	$(3.2\text{--}10) \times 10^{-12}$

height, γ is the ratio of the heat capacity at constant pressure to the gas constant, ρ is the atmospheric density, and ϕ is the upward heat flux carried by free convection. Taking $\phi \approx 10$ – $100\ \text{erg cm}^{-2}\ \text{s}^{-1}$ from ref. 26 yields $K \approx (5\text{--}10) \times 10^7\ \text{cm}^2\ \text{s}^{-1}$ at the 1,000 K level in our atmospheric model. These values suggest more sluggish convective mixing on Uranus than on Jupiter or Saturn where $K \approx 10^8$ – $10^9\ \text{cm}^2\ \text{s}^{-1}$ in the deep atmosphere^{6,16,27–29}.

Table 1 shows the predicted abundances of N_2 , CO, PH_3 , GeH_4 , HCN and CO_2 in the observable Uranus atmosphere for $K \approx 10^7$ – $10^8\ \text{cm}^2\ \text{s}^{-1}$. Specific kinetic calculations were not done for other potential non-equilibrium gases such as C_2H_6 , CH_3SH , CH_3NH_2 and CH_3OH , but quench temperatures of $\approx 1,000$ K may not be unreasonable for these gases³⁰, the resulting abundances can then be estimated from Fig. 1. Since precipitation of $NH_4Cl(s)$ and $NH_4F(s)$ may be very difficult to quench, HCl and HF are likely to be in equilibrium; however, if the low NH_3 mixing ratios ($< 10^{-6}$) inferred by Gulikis *et al.*²⁰ hold throughout the Uranian atmosphere there will not be enough NH_3 to react with the HCl and HF, so their equilibrium concentrations could be much higher than indicated in Fig. 1. Non-ideal gas behaviour may increase the pressure-dependent N_2 abundance; at our highest N_2 quench temperature (1,390 K), the pressure may be three times too high³¹ and thus the predicted N_2 abundance nine times too small, while at lower temperatures the required corrections becomes smaller.

Once the Voyager observations are in hand, the inverse process, namely deduction of K values from the observed abundances of PH_3 , GeH_4 and so on, will be possible using Fig. 2.

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Steam rapidly reduces the swelling capacity of bentonite

Rex A. Couture

Chemical Technology Division, Argonne National Laboratory,
 Argonne, Illinois 60439, USA

Sodic bentonite is widely used for industrial and scientific purposes, especially as a sealing agent¹, because of its great ability to swell in water^{2,3}. It has been proposed for use in high-level nuclear waste repositories as an impermeable barrier surrounding the waste package, or to fill tunnels, shafts and rooms. In some repositories, such as those planned in basalt, bentonite would be expected to be subjected to temperatures possibly up to 300 °C (refs 4, 5). As the waste would be approximately 600-900 m below the water table in fractured rock, the repository is expected to fill first with steam and then with liquid water^{4,6}. Reaction of bentonite with liquid water produces a minimal loss of swelling capacity, but I show here that reaction with water vapour at 150-250 °C results in rapid irreversible loss of most of the swelling capacity. This causes very large increases in permeability of sand-bentonite mixtures⁷, thereby reducing the ability of a bentonite barrier to retard the flow of groundwater in proposed high-level nuclear waste repositories.

The thermal and hydrothermal stability of bentonite has been repeatedly questioned^{8,9,10}, but transformations generally are expected to be slow enough and the duration of high temperatures brief enough that bentonite is not necessarily disqualified for use in a repository at high temperatures^{5,9,10}. Furthermore, in some environments, transformation may be limited by availability of potassium or other elements⁹⁻¹¹. In typical

hydrothermal experiments, montmorillonite was structurally unaltered, but underwent compositional changes after reaction with basaltic groundwater for 96 days at 360 °C, 30 MPa (ref. 10), and after reaction with basalt plus groundwater for 379 days at 300 °C, 30 MPa (ref. 5). Large reductions in the ion-exchange capacity¹² and small reductions in the swelling ability^{13,14} of bentonite were observed after heating to temperatures of 100-300 °C, but irreversible dehydration, which would imply a major mineralogical change, was not observed after heating for 340 days at 370 °C (ref. 5).

On the other hand, rapid and severe reduction in the swelling ability of bentonite because of exposure to steam has recently been observed⁷. In a series of experiments the permeability of sand-bentonite mixtures (25% bentonite, 75% basalt or quartz sand, with dry bulk densities of ~1.6 g cm⁻³), as measured by flow of liquid water, increased by up to 10⁵ as a result of prior exposure to water vapour at temperatures between 250 °C and 260 °C (ref. 7). The increase in permeability was due to a large, irreversible decrease in the swelling capacity of the bentonite. Here I present data on the effects of water vapour on bentonite at temperatures between 150 °C and 250 °C.

The methods and results are shown in Table 1. The untreated bentonites swell to very high specific volumes in water. For example, Envirolgel expands to a specific volume of 37 ± 4 cm³ g⁻¹, and forms a thick gel at specific volumes of 30 cm³ g⁻¹ or less. The maximum specific volume in water is defined here as the swelling capacity.

Dry heating to 250 °C for 7 days with no added water seems to reduce the swelling capacity slightly (sample 5), as observed previously^{13,14}. This reduction may have resulted from reaction with water of hydration before the sample dried, as the following experiments suggest.

Heating the bentonite to 250 °C in a closed system, with a limited amount of water, caused a great change in wetting and swelling behaviour. The unreacted material forms a sticky, gummy paste when mixed with water, but the reacted material is not cohesive in water, and has greatly reduced swelling capacity (samples 6-11, 19-21). All four bentonites were affected similarly. Bentonite initially contains about 8% water of hydration; even this amount of water, if retained during heating, is enough to severely alter the swelling properties of the bentonite

Table 1 Effects of heat and water vapour on swelling capacity of bentonite

Sample no.	Bentonite	Mass of bentonite (g)	Water added (g)	Water composition	Temperature (°C)	Total water bentonite	Swelling capacity (cm ³ g ⁻¹)
1	Envirolgel	—	—	—	Untreated	—	37
2	SWy-1	—	—	—	Untreated	—	37
3	Montmorillonite 27	—	—	—	Untreated	—	39
4	National Standard	—	—	—	Untreated	—	25
5	Envirolgel	4.84	0	dw	250	0	28
						(heated dry)	
6	Envirolgel	4.84	0	dw	250	0.08	9
7	Envirolgel	4.84	0.10	dw	250	0.10	9
8	Envirolgel	4.84	0.19	dw	250	0.12	7
9	Envirolgel	4.84	0.34	dw	250	0.15	4
10	Envirolgel	4.84	0.82	gw	250	0.25	5
11	Envirolgel	4.84	0.82	dw	250	0.25	5
12	Purified Envirolgel	1.00	0.20	dw	250	1.08	7
13	Envirolgel	2.00	2.12	gw	250	1.14	10
14	Envirolgel	0.25	5.00	dw	250	20†	>20†
15	Envirolgel	0.25	5.00	gw	250	20†	>20†
16	Altered Envirolgel	0.25	5.00	gw	250	20	4
17	Envirolgel	4.84	0.34	dw	200	0.15	9
18	Envirolgel	4.84	0.34	dw	150	0.15	13
19	SWy-1	4.46	0.29	dw	250	0.14	5
20	Montmorillonite 27	4.84	0.34	dw	250	0.15	13
21	National Standard	4.84	0.34	dw	250	0.15	11

Samples of bentonite were reacted with deionized water (dw) or basaltic groundwater (gw) for 5-7 days (5 days for samples 5 and 11; 7 days for the others) in small stainless steel or Inconel autoclaves. The composition of the groundwater was 3.0 mM NaCl, 2.0 mM Na₂SiO₃, 2.0 mM NaF, 1.1 mM Na₂SO₄, 0.7 mM NaHCO₃, 0.6 mM Na₂CO₃, 0.03 mM CaSO₄, adjusted to pH 9.9 with HCl. The autoclave volumes were 7.6-7.9 cm³, except for sample 20 (5.4 cm³). After reaction, the swelling capacity of the clay was determined by dispersing 0.10-0.15 g (dry weight) of clay ultrasonically with 4 ml of water in graduated conical centrifuge tubes at ~22 °C, allowing the clay to settle and measuring the expanded volume. The expanded volumes did not change after several months of standing. The error in swelling capacity is ±10%. The temperature accuracy is ±5 °C. The bentonites were from the following sources: Envirolgel, Wyo-Ben, Inc.; SWy-1, Clay Minerals Society; Montmorillonite 27, Ward's Natural Science Establishment, similar to American Petroleum Institute Montmorillonite 27; National Standard, Baroid Corporation. Except for samples 12 and 16, the only pretreatment of the bentonite consisted of drying, crushing and sieving by the suppliers. Except for quartz and plagioclase, the samples were nearly pure montmorillonite, with sodium as the predominant exchangeable cation. Smaller amounts of potassium feldspar (probably sanidine), illite or mica, calcite, gypsum and apatite were also present. Most of the experiments were done with Envirolgel (Wyo-Ben, Inc.), which contains ~80% montmorillonite and is described elsewhere¹⁰. Purified Envirolgel (sample 12) was prepared by sedimentation in water to remove about 20% sand and silt. Altered Envirolgel (sample 16) was prepared by previous reaction for 6 days at 250 °C, water/bentonite ratio = 0.15.

* Includes (8 ± 4)% initial water content of bentonite, except for sample 5, which was heated dry in an open vessel.

† Water and bentonite mixed ultrasonically before heating. Sample absorbed all water to form a gel, and remained gelled after hydrothermal treatment.

Table 2 Alteration of bentonite at controlled P_{H_2O}

Bentonite	Temperature (°C)	P_{H_2O} (MPa)	Relative humidity (%)	Swelling capacity ($\text{cm}^3 \text{g}^{-1}$)
Envirogel	250	3.7	93 ± 2	10 ± 1
Envirogel	Unaltered	—	—	37 ± 4

Bentonite was heated to 250 °C in a vessel at atmospheric pressure, the vessel was evacuated, water vapour introduced at $P_{H_2O} = 3.7$ MPa, the pressure was maintained for 18 h, and then the pressure was released, the bentonite was cooled to room temperature and the swelling capacity was determined. Liquid water was never present during alteration; the maximum P_{H_2O} was $93 \pm 2\%$ of saturation. (The temperature was controlled by a mechanical convection oven to a precision of better than ± 0.1 °C. P_{H_2O} as a percentage of saturation pressure was measured with a high-precision transducer and was verified by measuring the vapour pressure of pure water at 250 °C.)

(sample 6). Reaction with basaltic groundwater has the same effect as reaction with pure water (samples 10, 11), because the concentration of solutes in the water is insignificant compared with the concentration of soluble impurities and exchangeable ions in the bentonite.

Measurements of vapour pressure and water content of bentonite (unpublished work) have shown that the water is partitioned between water of hydration and vapour. Liquid water did not coexist with these samples at 250 °C.

To clarify the effects of liquid, vapour and water of hydration on bentonite, two experiments were performed, one in which liquid water was always absent and one in which liquid water was always present (to form a gel). In the first experiment, the bentonite was altered isothermally at a controlled partial pressure of water, 93% of saturation. Table 2 gives methods and results. The great reduction in swelling capacity shows that water of hydration and/or vapour was responsible for alteration. In the second experiment, water and bentonite were mixed in a ratio of 20:1 to form a gel. After reaction (samples 14 and 15, Table 1), the samples remained gelled, showing little or no loss of swelling capacity. Thus, reaction with water vapour, but apparently not with liquid, causes rapid, severe loss of swelling capacity.

The maximum alteration took place at water/clay ratios of roughly 0.15–0.25 at 250 °C (Table 1). This corresponds approximately to one to three absorbed water layers per 10-Å silicate layer in the montmorillonite structure (with allowance for up to 0.12 g water vapour in the autoclaves). Evidently, some water is necessary for the alteration, but alteration is slow or does not

Table 3 Exchangeable ions in bentonite (mmol g^{-1}) before and after alteration

	Na (method 1)	Ca (method 1)	Ca (method 2)	Mg (method 1)
Untreated (purified Envirogel)	0.56	0.16	0.11	0.05
Treated (sample 12)	0.51	0.13	0.12	0.01

Method 1: Determined by leaching overnight with 1 M NH_4Cl . Solutions were analysed by inductively coupled plasma atomic emission spectroscopy (ICP). Analytical accuracy $\sim \pm 5\%$. Partial dissolution of calcite may increase Ca analyses. Method 2: Determined by washing with 60% ethanol in water (by volume) and then leaching with 0.5 M LiCl , 0.5 M CsCl solution in 60% ethanol (after Neal)¹⁷. Samples (0.15–0.21 g) were rinsed twice for 15 min each with 60% ethanol, leached for 12 h with LiCl , CsCl solution, washed with 10 ml ethanol/water, leached for 2 h with 10 ml LiCl , CsCl solution, washed with 10 ml ethanol/water, then 5 ml. Samples were centrifuged after each washing or leaching. Leach solutions were combined and analysed by ICP. All solutions were de-aerated and kept under N_2 before use. This method is reported to dissolve insignificant amounts of calcite¹⁷. Analytical accuracy $\pm 5\%$.

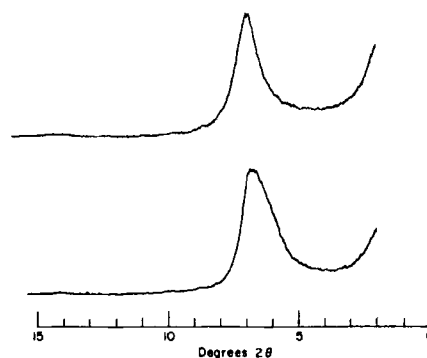


Fig. 1 X-ray diffraction of purified montmorillonite (Envirogel), unaltered (top) and altered (sample 12, bottom). Relative humidity controlled at 33% with saturated solution of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$. Basal spacings: 12.4 Å unaltered, 12.5 Å altered, determined from four basal reflections. Complete conversion to the calcium form would shift the spacing to 15.4 Å, at $5.7^\circ 2\theta$ (ref. 18). $\text{CuK}\alpha$ radiation, General Electric diffractometer, slits: 1° entrance, medium resolution divergence, 0.2° receiving, $2^\circ 2\theta \text{ min}^{-1}$, time constant 2 s.

take place in dilute systems, with low concentrations in the hydrated layers.

To determine whether the effect is reversible on contact with liquid groundwater, altered bentonite was reacted with a large excess of liquid groundwater. The swelling capacity of the product (sample 16) remained low. Therefore, the effect is not reversible over the timescale of the test.

The alteration is temperature-dependent, as shown by experiments at 250 °C, 200 °C and 150 °C (samples 9, 17 and 18). A large effect was observed as low as 150 °C.

The reduced swelling capacity corresponds to reduced uptake of water by hydrated layers of the montmorillonite structure. Unaltered montmorillonite expands greatly, in some cases from an average basal spacing of 10 Å in the dry state to over 100 Å in water^{2,15,16}. However, montmorillonite altered by water vapour at 250 °C expands only to a maximum basal spacing of 19 Å, which corresponds to three water layers per 10 Å silicate layer in the structure.

Except for the change in swelling behaviour, relatively little mineralogical change was observed. Both the altered and unaltered bentonite consist mainly of a dioctahedral smectite with an X-ray diffraction peak at 1.50 Å, and a basal spacing of 17 Å when treated with ethylene glycol. The reacted sample shows evidence of slight uptake of exchangeable calcium from calcite, as shown by energy dispersive analysis in the scanning electron microscope (SEM) and as suggested by a small X-ray reflection at $\sim 14.7^\circ$ (at 33% relative humidity). However, a sample which was purified by sedimentation before reaction showed a similar reduction in swelling capacity (sample 12, Table 1) but much less uptake of calcium, as determined by analyses for exchangeable Na and Ca (Table 3), by analysis of single particles in the SEM, and as suggested by X-ray diffraction at controlled humidity (Fig. 1). Evidently, the change in swelling behaviour is not due to extensive exchange of calcium for sodium. Alteration was accompanied by significant fixation of magnesium (Table 3), but it is questionable whether the rather small amount of magnesium can account for the change in behaviour.

The mechanism has not been determined, but it may be due to hydrolysis of exchangeable cations, to an attack on the octahedral layer, with release of aluminium into interlayer positions, or to oxidation of ferrous iron.

The alteration was apparently not due to reaction with the vessel materials. No reaction rim was observed, and minimal swelling capacity was observed after reaction in either stainless steel or Inconel vessels. Furthermore, no uptake of Ni or Cr was detected by elemental analysis in the SEM.

The observed rapid change in montmorillonite properties has important implications for the use of bentonite at high temperatures. If bentonite is exposed to moisture at temperatures

of 250 °C or higher, the permeability⁷ and the ability to swell and fill fractures will be greatly decreased. The effects observed here need to be addressed for safe design of nuclear waste repositories, and additional work on the mechanism of alteration may be required. Reduction of the swelling capacity also occurs at 150 °C and may occur at still lower temperatures, especially after long time periods. Bentonite used to fill tunnels and drifts in nuclear waste repositories may reach high enough temperatures to be adversely affected by water vapour.

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Spin-dependent electron scattering from optically active molecules

D. M. Campbell & P. S. Farago

Department of Physics, University of Edinburgh, Edinburgh EH9 3JZ, UK

The origin of the isomeric purity of biomolecules has been extensively discussed¹. In particular, it has been suggested that spin-polarized electrons or positrons emerging in the β -decay of radioactive nuclei may have led to a preferential destruction of one of the two isomers at an early stage of evolution and that this process tipped the initial balance of abundance of the two self-replicating isomers². As experimental tests of this hypothesis have remained largely inconclusive^{3,4}, it seemed useful to investigate the spin dependence of electron scattering from optically active molecules under the simplest possible conditions. Here we describe an experiment which indicates that spin-polarized electrons, like polarized light, when scattered from optically active molecules, can 'distinguish' between right-handed and left-handed isomers.

The application of fundamental conservation principles has shown that the 'handedness' of target molecules leads to spin-

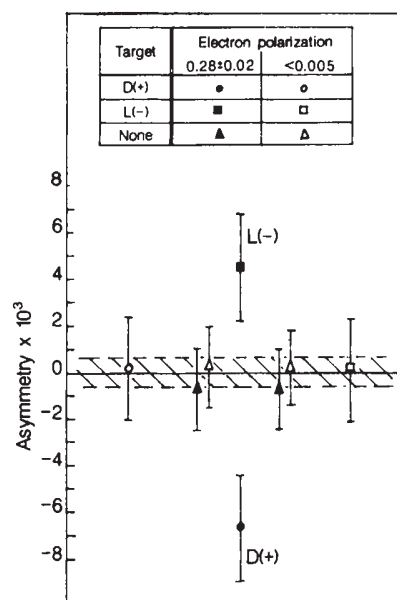


Fig. 1 Measurements of the electron polarization and beam attenuation with and without camphor and alternating the load in the target cell between the D(+) and L(-), respectively. Those obtained with camphor using polarized incident beams ($P = 0.28 \pm 0.02$) are marked D(+) and L(-), respectively. The remaining points were obtained with unpolarized incident beams ($P < 0.005$) or without camphor. The error bars indicate 1 s.e. The results obtained with unpolarized electrons or without camphor were also combined. The standard error of their weighted mean defines the hatched belt between the broken lines.

dependent scattering phenomena that are completely analogous to optical activity and circular dichroism⁵. Our objective here was to measure the attenuation of longitudinally polarized electron beams passing through a vapour of optically active molecules. This attenuation can be calculated in terms of two elastic forward-scattering amplitudes, one spin-independent, f , and the other chirality-dependent, h . The sign of h changes with the handedness of the molecules; $h = 0$ for non-chiral molecules. If I_0 is the incident beam intensity, P its longitudinal polarization, ρ the vapour density, z the path length, and the electrons undergo single scattering only, then the transmitted intensity is

$$I = I_0 \exp(-\rho qz) [\cosh(qpzX) - P \sinh(qpzX)] \quad (1)$$

where $q = (4\pi/k) \text{Im}(f)$ is the total spin-independent scattering cross-section and $X = \text{Im}(h)/\text{Im}(f)$. X measures the chirality-dependent cross-section in units of the spin-independent one, I_m denotes the imaginary part and k is the electron wave number.

If the transmitted intensity is measured for a pure isomer at a given target density and a given electron polarization, alternately parallel and antiparallel to the beam axis, the two output intensities, $I(P)$ and $I(-P)$, yield an intensity asymmetry,

$$A(P) = [I(P) - I(-P)]/[I(P) + I(-P)] \\ = -P \tanh(qpzX) = -PqzX \quad (2)$$

to first-order approximation.

Thus, the following predictions can be made. (1) For a given isomer at a fixed magnitude of the longitudinal electron polarization, the magnitude of the asymmetry increases linearly with the sample density. (2) The asymmetry changes sign if the handedness of the sample is reversed. (3) The asymmetry vanishes if either the electron beam is unpolarized or the sample is a racemic mixture.

The first experiment, designed on the grounds of general symmetry considerations and using D(+)-camphor molecules as target and 25 eV initially unpolarized electrons, remained inconclusive in that it could establish only an upper limit to the in-plane polarization of the scattered electron⁶. Gidley *et al.*⁷ investigated asymmetrical positronium formation in optically

active molecules using slow spin-polarized positrons and found a positive effect in one case but none in two others.

The apparatus used in the present experiment consists of three stages: a GaAs polarized electron source⁸, an electron polarimeter used in a transmission mode to monitor the incident beam intensity and electron polarization⁹, and an electron spectrometer, adapted from the scheme introduced by Simpson¹⁰. The scattering target was camphor vapour at room temperature, and the experiment was carried out at 5 eV energy. Simultaneous measurements of the electron polarization and beam attenuation consisted of 'runs' taken in turn with and without camphor and alternating the load in the target cell between the D(+) and L(-) isomer, respectively. The results displayed in Fig. 1 were obtained by combining the results of corresponding runs.

Figure 1 shows, first, that in the absence of camphor and in the case of unpolarized incident electrons the observed asymmetry vanishes and, second, that for the two isomers, when electrons beams of the same polarization are used, the asymmetry is of different sign. The measured values are different from zero by 2 s.e. or more; the magnitudes of the asymmetries agree within their error margin. Thus, we conclude that the results confirm the existence of an 'electron optic dichroism' with a confidence limit exceeding 95%.

The experimental results allow a tentative estimate of the ratio $Im(h)/Im(f)$. In typical working conditions, camphor in the sample cell attenuated the beam by a factor of 4-5. As our equipment could not give an absolute measurement of the sample pressure, only relatively crude tests were performed to see that up to this level of attenuation single scattering conditions prevailed. Thus, from equation (2)

$$A = -P \ln(I_0/I) Im(h)/Im(f)$$

and hence, with the measured values $P = 0.28$, $4 < I_0/I < 5$ and $A > 10^{-3}$, one finds

$$X = Im(h)/Im(f) > 2 \times 10^{-3}$$

The mechanism of the interaction involved is unknown. There are no theoretical estimates of X , although there are estimates of the related quantity $Re(h/f)$, where Re denotes the real part. Based on the 'helicity density' model, Rich *et al.*¹¹ estimated for camphor at low electron energy $Re(h/f) \sim 10^{-5}$. Hayashi¹² calculated the in-plane component of the spin polarization of initially unpolarized electrons scattered elastically by optically active molecules (1,3-dimethylallene) at 50 eV and above: $Re(h/f) < 5 \times 10^{-7}$.

We stress that the ratio X can be significantly different from $Re(h/f)$. More importantly, neither of the theoretical calculations quoted takes account of possible resonance scattering, which may increase the effect by orders of magnitude. At the energy at which the present experiment was conducted (5 eV), the possibility of resonance effects cannot be ruled out. It is suggestive that the optical absorption of photons about this energy certainly involves pronounced broad resonance^{13,14}.

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A small coleoid cephalopod with soft parts from the Lower Devonian discovered using radiography

W. Stürmer

Private Research Laboratory for Interdisciplinary Palaeontology, Burgbergstrasse 20, D-8520 Erlangen, FRG

During the past 50 years, X-ray examinations have greatly increased our knowledge of fossils. Modern radiographic methods, X-ray tubes with fine focal spots, high-resolution films and optimal exposure conditions have revealed details of the anatomy and general biology of ancient life as well as species and representatives of major groups hitherto unknown in the Lower Devonian¹⁻⁵. Most of this material came from the Hunsrück slate, where most fossils are converted to pyrite, FeS₂, giving a good contrast in the radiographs. Additional image processing may reveal even more details. While screening large quantities of black slate pieces, I have discovered a small teuthid coleoid showing soft parts (Fig. 1) which is the best-preserved specimen I have found during 20 years of investigation. This *Eoteuthis elfriedae* n.sp. is very similar to the living *Alloteuthis africana* of the Loliginidae, and is one of many unusual fossils yielded by this slate³⁻⁵, only a few of which have been published. This specimen of *E. elfriedae* shows that *Alloteuthis*-like animals have not changed much over the past 400 Myr, and means that previous concepts of the appearance of such forms must be revised.

More than 100 gladii and cuttle bones of other teuthid coleoids have been recovered in the past 2 years from the Kaisergrube, an old mine near Gemünden in the Hunsrück. They all show a typical growth striation, but radiography reveals that none of them has soft parts. Similar striation has been observed⁶ in the

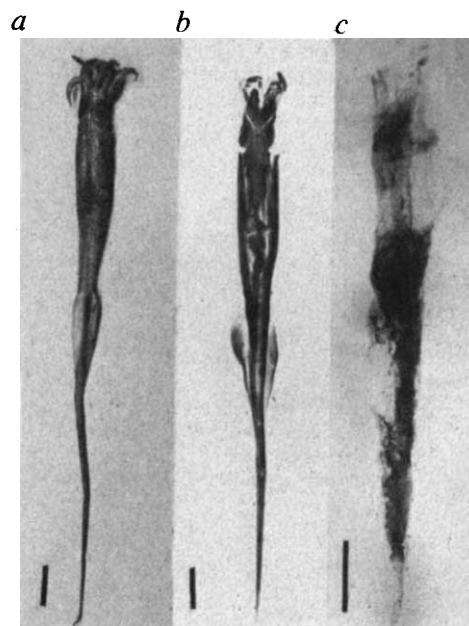


Fig. 1 a, Extant *Alloteuthis africana* (Gulf of Guinea, R/V Geromino Sta 2-730680, Smithsonian Institution). b, Radiograph of *A. africana* (Gulf of Guinea, R/V Geromino Sta 2-1910 from 730680, Smithsonian Institution). c, Radiograph of the Lower Devonian teuthid of the Black Slate of Bundenbach/Hunsrück, FRG (WS 3023). Scale bars, 10 mm. The radiographs were made with a Siemens Kristalloflex II unit in combination with a special water-cooled fine focus tube. The Agfa Millimask plates were used as photographic material which allow observation of details down to $< 5 \mu\text{m}$. Intermediate negatives with enhanced detail contrast were made using a TV-microscope in combination with a Siemens Transicon image processing unit.

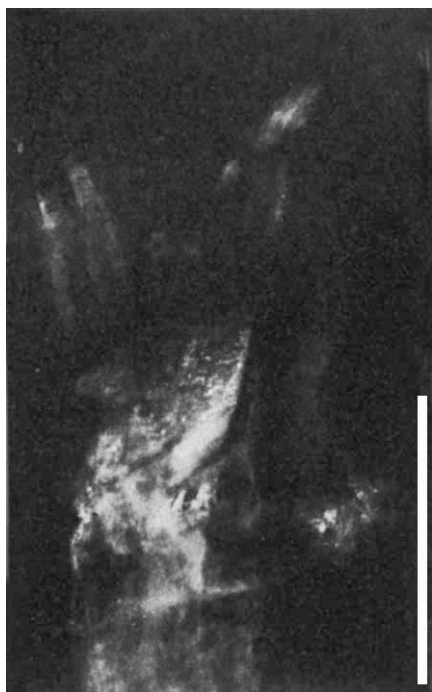


Fig. 2 The head of *Eoteuthis* showing the tentacles. The series of pyrite dots is visible. The jaw can be seen in the lower half. Scale bar, 10 mm.

Table 1 Description of *Eoteuthis elfriedae* n.sp.

Class	Cephalopoda
Subclass	Coleoidea BATHER 1888
Order	Teutkida NAEF 1916
Family	Loliginidae <i>sensu stricto</i> Steenstrup 1861. As only one specimen exists, the characterization of the genus must be the same as that of the species
Species*	<i>Eoteuthis elfriedae</i> n. sp.
Holotype	Bavarian State Coll. Munich, No. 1984 I 100 (Radiographs: WS 3023); specimen showing soft parts on radiographs (Fig. 1)
Locality	Black slate mine Herrstein, Bundenbach, Hunsrück (FRG)

* The species is named in honour of Mrs Elfriede Stürmer, whose enthusiastic efforts have revealed hundreds of Lower Devonian coleoid cephalopod remains.

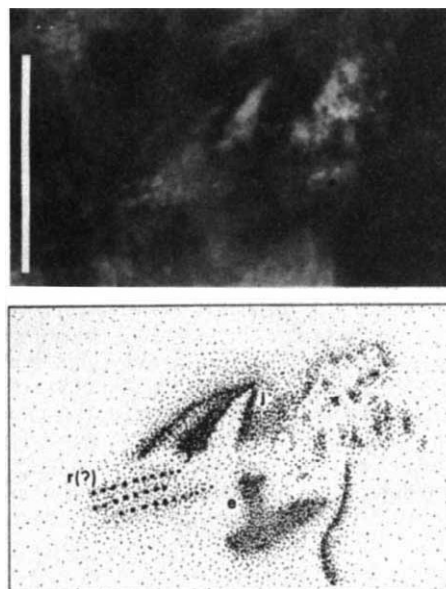


Fig. 3 The jaw of *Eoteuthis* after image processing: j, jaw; e, oesophagus; r, radula (?). Scale bar, 1 mm.

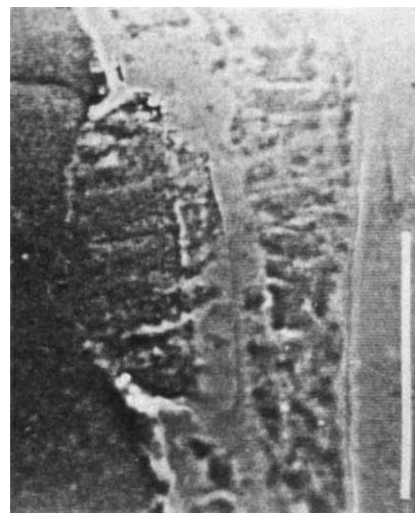


Fig. 4 The fin of *Eoteuthis* with concave posterior border after electronic image processing. Scale bar, 10 mm.

Table 2 First known evidence of cephalopod fossils

Cambrian	Ordovician	Silurian	Devonian	Carboniferous	Permian	Triassic	Jurassic	Cretaceous	Tertiary
							①		
				②					
			③						
④									

1, *Proteroctopus ribeti*; 2, *Jeletzkyia douglassae*; 3, *Eoteuthis elfriedae*; 4, Orthoceratid cephalopods.

fragmental prebelemnites, *Hyolithoconularia striata* found in the Lower Devonian of Morocco. A detailed account on the Hunsrück material will be published elsewhere.

The preservation of the soft part of a fossil is unusual but the Hunsrück slate contained favourable conditions for preservation^{4,5}: the organic sulphur compounds of the animal (or of sulphur bacteria) form pyrite, FeS₂ in the presence of iron ions in the interstitial water of the sediment. This pyrite is opaque

to X rays, so even a thin veil of FeS₂ at optimal exposure conditions produces a good contrast in the radiograph^{4,5}.

The body organization of this little teuthid, *E. elfriedae*, resembles that of the living *Alloteuthis* (Table 1) and seems to indicate that this type of cephalopod existed in the Lower Devonian. It is a good example of long survival in the fossil record.

This small teuthid cephalopod is similar to the extant *A.*

africana, with some well-preserved soft parts. There are eight or nine visible arms/tentacles, not definable in detail; the jaw is partly visible in the radiograph (Fig. 2) together with a fin which has a concave posterior border (Fig. 4). The total length of *E. elfriedae* is 83 mm, its maximum width 9 mm. The specimen is flat and has an estimated thickness of <0.5 mm; the original diameter of the more or less tubiform animal may have been ~6 mm. Some of the arms/tentacles have rows of little spots (suckers?) (Fig. 2). The jaws are visible just behind the tentacle bases (Fig. 3). The oesophagus seems to be below the tip of the beak (Fig. 3, e). To the left, between the beak and oesophagus are three rows of faint regular dots which may represent the radula (Fig. 3, r). One of a pair of lateral fins (Fig. 4) has a concave posterior border, as in the extant *Alloteuthis*⁷. The opposite fin is directly attached to the body and is thus invisible in the radiograph. Owing to the heavy pyritization of the body the gladius cannot be reconstructed. We must assume that the animal was buried very rapidly which prevented bacterial dissolution of the soft structures. Also, compaction must have taken place at an early state of diagenesis, flattening the body and the soft parts to <0.5 mm. The distortion of the soft parts as a result of this compaction makes it difficult to compare with living *Alloteuthis*-like animals. The process of pyritization is not fully understood. Observations on slate pieces from the Kaisergrube reveal hundreds of small cephalopods and tentaculites on one slab, and several states of FeS₂ exist side by side. My experiments on the fossilization process in the laboratory by putting *Cirolana* specimens from South Norway into different metal salt solutions produced a surprising result: radiographs made at 6-weekly intervals for the first year, then at 6-monthly intervals, show a 'fossilization' that remains constant after the first 6 weeks, so the experiment was concluded after 4 yr.

There are many tables⁸⁻¹² illustrating the ancestry of cephalopods, but because some points in such evolution charts are based on only a few moderately well preserved fossil specimens, only general statements should be made until we know more about fossil and modern gladii (see ref. 13 for a critical analysis). Table 2 shows that a fully developed octopus, *Proteroctopus tibeti*¹, existed in the Jurassic; Johnson and Richardson² found a 10-armed cephalopod *Jeletzkyia douglassae*, in the Mazon Creek fauna from the Middle Pennsylvanian of Illinois. Radiography clearly reveals the complete tentacular crown and fragile shell of this unique fossil. Double rows of hooks on the arms are well preserved. *E. elfriedae* is a highly developed teuthid of the Lower Devonian, and the ectocochleate nautiloids go back to the Cambrian¹⁴. If we consider the timescale and the fact that the fossil record shows few closely related forms, then more questions arise, especially as to the origin of these animals. How long did it take to evolve from a primitive nautiloid to *Eoteuthis*, and where might one hope to find connecting forms?

I thank Professor G. L. Voss for the first hint that the fossil may be an *Alloteuthis* and for helpful discussions. Financial support for the Hunsrück research by the Volkswagen Foundation and the German Research Society is acknowledged. I also thank Dr C. F. Roper and Dr Jeletzky, for many discussions; and especially Dr C. F. E. Roper for specimens of extant *Alloteuthis* animals for comparison radiographs.

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Biogenic fluxes of carbon and oxygen in the ocean

Trevor Platt & William G. Harrison

Marine Ecology Laboratory, Bedford Institute of Oceanography, PO Box 1006, Dartmouth, Nova Scotia, Canada B2Y 4A2

Rates of oxygen utilization (OUR) at depth in the ocean have been interpreted as showing that rates of carbon fixation by phytoplankton, as estimated by ¹⁴CO₂ assimilation *in vitro*, must be in error¹. The oxygen is consumed in the decomposition of organic matter sinking from the photic zone: there is a stoichiometrically equivalent flux of nitrate from the deep water towards the surface². For comparison with the ¹⁴C data, it is conventional to extrapolate OUR to total equivalent phytoplankton production through a constant factor *f*, the ratio of nitrate-based production (*P_{new}*) to total production (*P_t*) as defined by Dugdale and Goering³. The alternative hypothesis, that scaling up OUR by a constant factor *f* is inappropriate, has not been examined in detail. We show here that *f* is variable in space and time for most provinces of the ocean. Furthermore, we show that in nitrogen-limited systems, such as the pelagic of the open ocean, *P_t* and *f* should be positively correlated. Applying these results to data from the Sargasso Sea, we find that the carbon fluxes estimated by ¹⁴C assimilation are consistent with the oxygen fluxes estimated by OUR. The conclusion is of profound importance for understanding the major biogeochemical cycles of the ocean.

Alternative methods for measuring the same ecological rate do not necessarily refer to the same time-scale. Accumulation of oxygen in the photic zone of the ocean⁴ or its consumption at depth^{1,5} provide estimates of phytoplankton production with a characteristic timescale of months to years. Measurements of carbon fixation by *in vitro* incubation have a characteristic time-scale of the same order as the incubation time, ≤24 h. We have pointed out^{6,7} that extreme caution should be taken when comparing data sets whose intrinsic timescales are not compatible. The one has to be averaged to conform with the other, and we usually have little information on which to base the averaging in time. Moreover, OUR calculations imply some averaging over depth, whereas ¹⁴CO₂ assimilation is measured at discrete depths.

For an arbitrary depth stratum, one need not necessarily expect that the carbon and oxygen fluxes will balance when averaged over some arbitrary time interval. Within the variance between years (which can be appreciable⁸), the natural biological timescale at which the carbon and oxygen fluxes can be expected to balance is the annual one. One of the very few

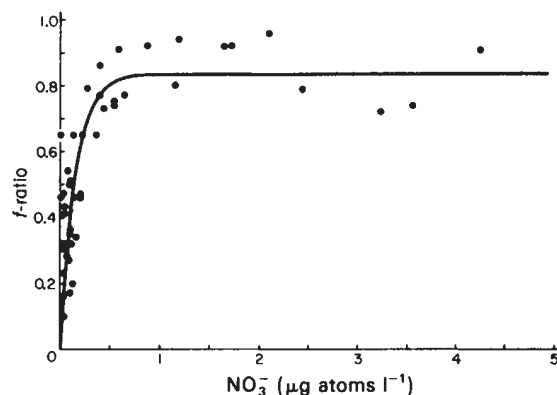


Fig. 1 Relationship between *f* and ambient NO₃⁻ concentration using the depth profile data of ref. 25 and the seasonal data of ref. 26. Data for which ambient NH₄⁺ concentrations exceeded 0.1 μg atoms l⁻¹ were omitted. Data fitted to an exponential model: $f = f_{\max}(1 - e^{-(\alpha \text{NO}_3^-/f_{\max})})$, for which $f_{\max} = 0.83 \pm 0.08$ and $\alpha = 5.48 \pm 0.77$.

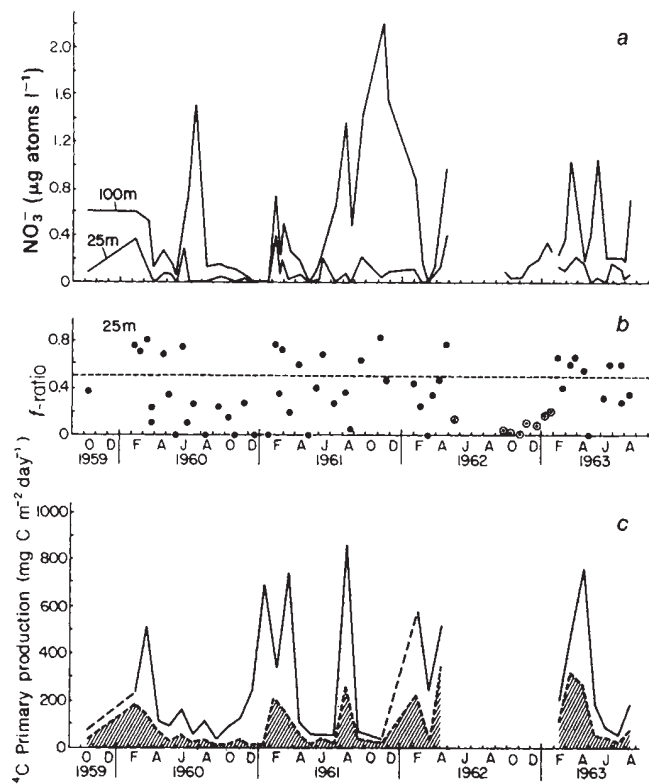


Fig. 2 Time-series data of: *a*, ambient NO_3^- concentrations; *b*, f computed from the model given in Fig. 1; and *c*, total ^{14}C , and new primary production (P_{new}) at station S, Bermuda; integrated 0–100 m. New production (P_{new}) estimated as fP_T where P_T is assumed to be equivalent to ^{14}C results. In *b* $\odot$ are f values determined directly from ^{15}N measurements (ref. 3); these data appear to be fully consistent with the trends shown by the indirectly determined values of f .

oceanic data sets having anywhere near sufficient resolution in time that reliable annual means of primary production and OUR can be calculated is that from station S in the Sargasso Sea⁸. From these data, Jenkins and Goldman⁵ compute an OUR at depth of the order of $5 \text{ mol m}^{-2} \text{ yr}^{-1}$. We now consider how this estimate of new production should be scaled up to give total production.

The conventional procedure¹ is to apply to the annual new production a constant factor $\bar{f} \approx 0.05$ said to be characteristic of oligotrophic ocean water². An alternative procedure, adopted here, is to estimate $f(z, t)$ for each depth z for all times t during the year.

Normally f is estimated from measurements of the *in vitro* assimilation rates of $^{15}\text{NO}_3$ and $^{15}\text{NH}_4$. There are very few such data for the Sargasso Sea. However, provided the concentration of ammonia is low, $\leq 0.1 \mu\text{M}$, such that neither the uptake of nitrate nor its enzymatic reduction are suppressed, f can be related to ambient nitrate concentration (Fig. 1). We use this dependence to estimate $f(z, t)$ from the nitrate concentrations reported at station S (Fig. 2). It is found that f is not constant, but varies between 0 and 0.84.

We now state an important inequality for nitrogen-limited systems:

$$\iint f(z, t) P_T(z, t) dz dt \geq \bar{f} \iint P_T(z, t) dz dt \quad (1)$$

where

$$\bar{f} = \frac{\iint f(z, t) dz dt}{\iint dz dt}$$

In other words, P_{new} estimated by the weighted integral on the left-hand side of equation (1) will always exceed P_{new} estimated

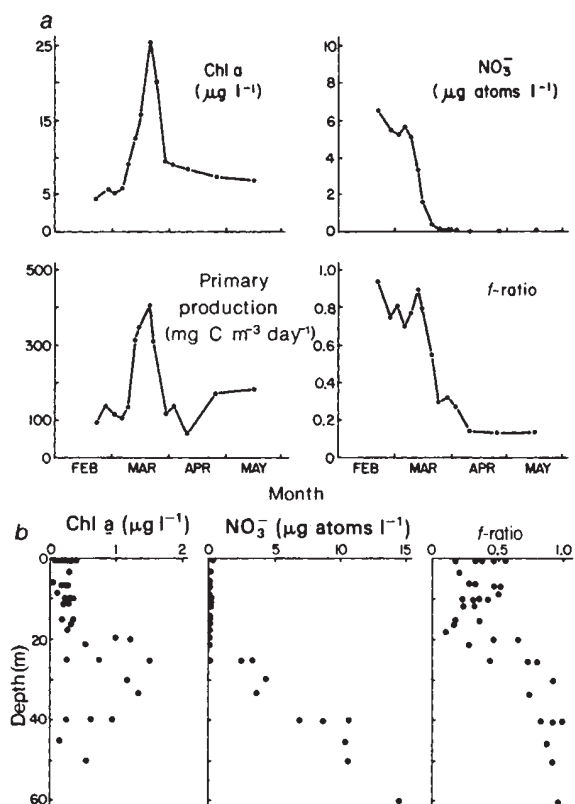


Fig. 3 Relationship between f and ambient NO_3^- concentration: *a*, during the decline of the spring phytoplankton bloom in a temperate coastal embayment (Bedford Basin, Nova Scotia); and *b*, in depth profiles on the continental shelf of the middle Atlantic Bight in summer. Here, f is derived directly from $^{15}\text{NO}_3^-$ and $^{15}\text{NH}_4^+$ uptake measurements.

by the right-hand side, mean value approximation. This is because $P_i(z, t)$ and $f(z, t)$ will have a finite (positive) covariance where nitrogen is the limiting resource. That is, a local pulse in available nitrate will result in a local increase in f at the same time²⁷ as it increases the local magnitude of P_i (Figs 2, 3).

Applying the weighted integral to the ^{14}C data for station S, one finds that, on an annual basis, new production is 31% of total production (Table 1). The ^{14}C data show an average production of $6.8 \pm 1.9 \text{ mol C m}^{-2} \text{ yr}^{-1}$, separable now into $2.1 \pm 0.58 \text{ mol C m}^{-2} \text{ yr}^{-1}$ of new production and $4.7 \pm 1.3 \text{ mol C m}^{-2} \text{ yr}^{-1}$ regenerated production ($P_T - P_{\text{new}}$). Applying photosynthetic quotients of 1.8 to the new production and 1.25 to the rest⁹, we find the oxygen equivalent of the ^{14}C results to be $9.7 \pm 1.9 \text{ mol O}_2 \text{ m}^{-2} \text{ yr}^{-1}$, of which $3.8 \pm 1.0 \text{ mol O}_2$ is new production. Jenkins and Goldman⁵ found an OUR at depth to be in the range $4.1\text{--}5.9 \text{ mol O}_2 \text{ m}^{-2} \text{ yr}^{-1}$ depending on the corrections applied for diffusive losses and abyssal respiration. It does not appear, therefore, that there is any significant difference between the two independent indices of new production. And we certainly do not find that the respiration at depth (P_{new}) exceeds the primary production at the surface (P_T), as was claimed for the subtropical gyre of the North Atlantic, and interpreted as showing that the ^{14}C results must be in error¹.

In fact, adopting a small and constant $\bar{f}$ when P_{new} is as high as $5 \text{ mol O}_2 \text{ m}^{-2} \text{ yr}^{-1}$ leads to a contradiction in Fig. 2a of ref. 2, for then the implied estimate of P_T is $\approx 100 \text{ mol O}_2 \text{ m}^{-2} \text{ yr}^{-1}$, which would require an asymptotically high value for $\bar{f}$ (≈ 0.5). We have shown that the contradiction stems from an improper selection of $\bar{f}$ rather than from a discrepancy between the ^{14}C results and the true values of total production. Indeed, putting the effective annual $f = 0.31$ as calculated here into equation (1) of ref. 2 yields $P_T = 124 \text{ g C m}^{-2} \text{ yr}^{-1}$, not grossly at variance with the mean annual ^{14}C value of $82 \pm 23 \text{ g C m}^{-2} \text{ yr}^{-1}$ (Table

Table 1 Mean monthly and annual ^{14}C primary production rates from Station S, 1959–63

Month	Primary production (g C per m ² per month)		
	P_T	P_{new}^*	f
1	12.2 (12.4)	1.2 (1.5)	0.10
2	10.2 (5.1)	5.4 (1.6)	0.53
3	14.9 (6.1)	4.8 (3.4)	0.32
4	11.3 (9.7)	5.5 (4.4)	0.49
5	3.3 (2.2)	0.8 (0.6)	0.24
6	2.8 (1.5)	1.2 (0.3)	0.43
7	1.7 (0.1)	0.6 (0.1)	0.35
8	11.8 (12.5)	3.5 (3.6)	0.30
9	1.6 (0.5)	0.7 (0.5)	0.44
10	2.5 (0.4)	0.6 (0.4)	0.24
11	2.3 (1.8)	0.7 (0.3)	0.30
12	7.5 (4.4)†	0.2 (0.1)†	0.03
Annual	82.1 (22.2)‡	25.3 (7.0)‡	0.31§

Monthly rate = mean daily rate $\times 30$. Data are given ± 1 s.d.

* $P_{\text{new}} = \int P_{\text{new}}(z, t) dz$ averaged for the month, where $P_{\text{new}}(z, t) = P_T(z, t) \times f(z, t)$.

† Variance estimated.

‡ The measures of dispersion given are ± 1 s.d. of the annual totals computed on the basis of the standard deviations of the monthly averages; they are not intended to be explicit statements of the confidence intervals at any particular probability level.

§ Annual P_{new} divided by annual P_T . Annual figures are based on some 51 sampling dates.

1). Note also that these two values are quite independent of each other.

Another problem with the extrapolation of high values of total production through division of new production by a small value of f is that the implied efficiency of photosynthesis is too great to accept. It can be shown that extrapolation of the OUR estimates of P_{new} to P_T through application of an f ratio ≈ 0.5 is equivalent to a photosynthetic efficiency $\approx 6\%$, a value too high to be believable as an annual average¹⁰.

Evidence is accumulating that the distributions of biological properties in the ocean are highly variable in space and time. We conclude this from sediment trap data¹¹; from satellite data¹²; from samplers deployed from a moving ship¹³; from theoretical physical oceanography¹⁴; and from theoretical ecology¹⁵. Superimposed on this variance in the Sargasso Sea is a strong seasonal cycle in both primary production and nutrient availability¹⁶: for example, the four months January–April account for $\approx 60\%$ of the annual assimilation of $^{14}\text{CO}_2$ (Fig. 4).

It therefore becomes increasingly untenable to speak about a particular ocean province, and certainly not the Sargasso Sea, as being 'oligotrophic'. Rather, a given oceanic province can be expected, locally in $(x, y, z$ and $t)$, to manifest a range of characteristics from apparent extreme oligotrophy to eutrophy. For example, judging by the f ratio alone, a temperate, coastal embayment can appear to change its trophic status from eutrophic to one characteristic of the open-ocean stereotype within the space of a few weeks, if a sufficiently detailed time-series is available (Fig. 3a). Local or transient fluctuations away from oligotrophy will account for a high proportion of the annual primary production. Moreover, most of the production in these events will be new production. The effective f ratio for the year (proportion of the total annual carbon fixation that is based on nitrate) will, therefore, be much higher than has previously been supposed.

The open ocean is still heavily undersampled with respect to primary production. For example, the water column primary production rates for Station S have to be computed from data collected at only three depths. In the open ocean, the distribution of phytoplankton is highly non-uniform in depth¹⁷, and the existence almost everywhere in the ocean of a deep chlorophyll maxima is well established¹⁸. If more sampling depths were

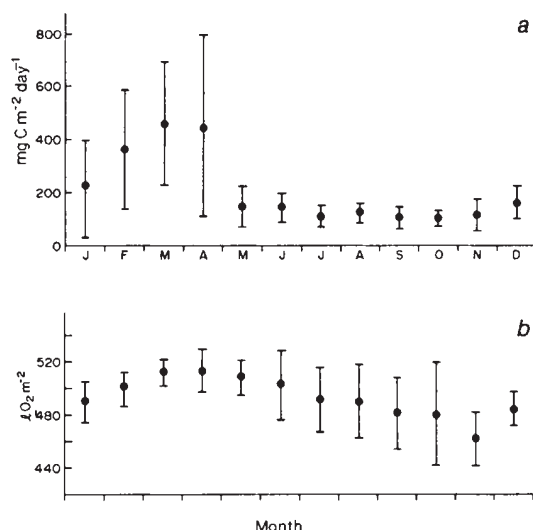


Fig. 4 Long-term averages (± 1 s.d.) of ^{14}C primary production (a) and dissolved O_2 (b) concentrations at Station S, Bermuda. These values represent a longer time-series (1957–63) than those shown in Fig. 2.

available, the estimate of depth-integrated production would probably increase. A similar tendency for the mean rates to increase can be expected as the resolution of sampling is improved in time and in the (x, y) plane.

We conclude that in a nitrogen-limited ocean ecosystem, local events (positive anomalies) in the nitrate field account for a disproportionately high fraction of the annual signal in primary production. During these events, the margin between new and total production narrows. On the other hand, the factor converting carbon fixation to oxygen evolution is at its highest. Taken together, these points are sufficient to show that the available flux data for carbon and oxygen in the Sargasso Sea are not internally inconsistent. It is one more example of the crucial importance of giving at least as much emphasis to the variances as to the means in discussing the dynamics of nonlinearly coupled systems¹⁹. The implied picture of the pelagic ecosystem (intermittent nitrate supply; new production one-third of total) is at variance with that presented by Goldman²⁰ where production is controlled by 'oases of regeneration' associated with particulate aggregates, and where 'spinning wheel' metaphor characterizes the supposed, rapid rate of nutrient recycling.

These conclusions are of importance not only for our understanding of the pelagic ecosystem but also for incorporation into budget calculations for the great biogeochemical cycles of the ocean^{21,22}. The conclusions have a direct bearing on current discussions of the possible role of the ocean biota as a control on the concentration of CO_2 in the atmosphere, and thus bear indirectly on the prediction of the future climate of the Earth^{23,24}.

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A structurally preserved magnolialean fructification from the mid-Cretaceous of Japan

Harufumi Nishida

International Budo University, 841 Monomizuka, Shinkan, Katsura, Chiba Prefecture, 299-52 Japan

Fossil evidence throwing light on the origin and diversification of angiosperms (flowering plants) has been increasing rapidly over the past 10 years. Most palaeobotanists accept that angiosperms first diversified in the Barremian stage of the Cretaceous, but this is based mainly on strato-phenetic morphological studies of palynomorphs and vegetative organs¹⁻³. Reproductive organs such as flowers and fructifications, which are vital to a full understanding of angiosperm evolution, are rare and have only recently been described in any detail from Cretaceous rocks⁴⁻¹¹, and even these are generally preserved as compressions or as fusinized remains. Here I report a permineralized angiosperm floral axis bearing conduplicate carpels from mid-Cretaceous sediments of Hokkaido, Japan. Its structure most closely resembles that of extant Monimiaceae and Austrobaileyaceae of the Magnoliales, particularly in the concave receptacle, but it shows several features considered more primitive than those occurring in any extant family. The fossil extends and clarifies knowledge of the structure and evolution of early angiosperms and is the first report of a permineralized angiosperm fructification of Cretaceous age.

The fructification was collected by Mr Masaharu Kasai in a tributary of the Sankebetsu River, Haboro district of Hokkaido, in Cretaceous shallow marine sediments of the Upper Yezo Group. The ammonite *Mesopuzosia pacifica* Matsumoto in the same calcareous nodule indicates a Turonian age.

The specimen has a woody peduncle, 25 mm long and 4 mm in diameter, with an attached globose floral structure ~25 mm in diameter (Fig. 1a). The floral structure is composed of many foliicles (Figs 1b, 2), and reconstruction (Fig. 2b) illustrates that

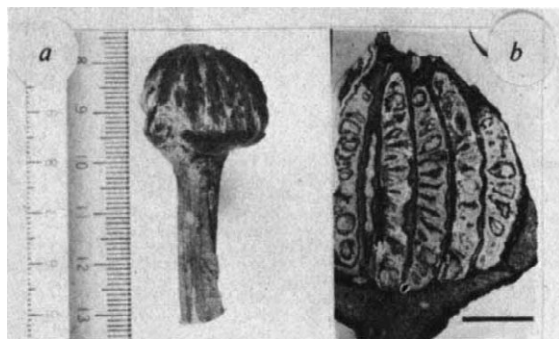


Fig. 1 a, Side view of the fossil fructification showing attachment to a long peduncle. Scale in cm. b, Longitudinal section showing concave receptacle and falcate foliicles containing ovules. Follicles from right to left correspond to those in Fig. 2b between the two parallel lines and numbered 18, 11, 6, 4, 2 and 3. Scale bar, 0.5 cm.

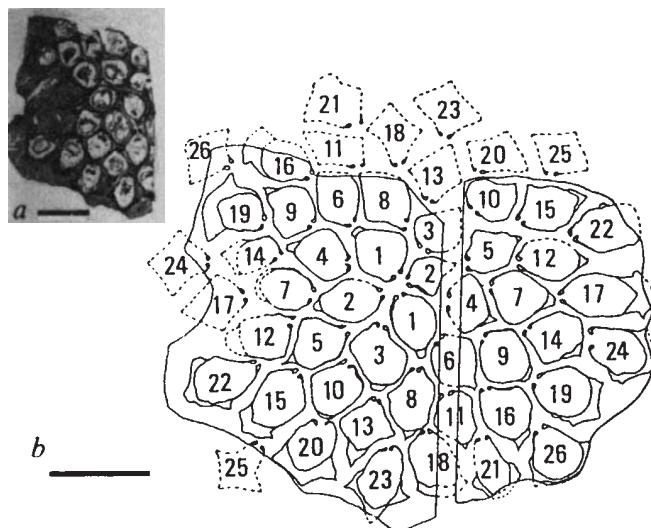


Fig. 2 Cross-sections of the fructification. The upper part of the figure corresponds to the surface shown in Fig. 1. a, Section of the specimen corresponding to the left half of b. b, Diagrammatic reconstruction of foliicle arrangement. Numbers show pairs of foliicles in a helically decussate arrangement. Scale bars, 0.5 cm.

the arrangement of the foliicles is intermediate between decussate and helical.

The median longitudinal section in Fig. 1b shows that the foliicles were borne on a concave receptacle. Each foliicle is 17 mm long $\times$ 3.1 mm wide and falcate, curved slightly towards the adaxial surface. They are sessile or have a short stalk and taper towards their apices. An adaxial longitudinal slit starts 1 mm from the base of each foliicle and runs nearly to the apex. A pair of marginal ribs occurs alongside this slit, bearing abundant unicellular, simple trichomes (Fig. 3). The slit evidently represents the stigmatic crest of a conduplicate carpel. Paracytic stomata occur on the external wall of the foliicle.

In the foliicle a pair of adaxial veins passes through the marginal ribs along the stigmatic crest. An abaxial vein runs right up to the apex of the foliicle, producing an unknown number of lateral bundles, and then curves to the adaxial surface, where it ends blindly about 0.5 mm below the point of incurving.

Most of the receptacle is infilled with sandstone matrix, but its base is preserved and shows many bi-collateral bundles arranged in a ring. There is no direct evidence that stamens or petals were attached, but bundles occur at the base of the receptacle which do not enter foliicles and are directed to the receptacle periphery. These may represent traces to stamens or perianths.

There are at most 15 ovules per foliicle attached alternately along the adaxial stigmatic crest, and therefore having marginal placentation. The funicles are short and the ovules bitegmic (Figs 3, 4). The outer integument comprises a thick, sclerotic outer layer and a thin inner one which develops a thick parenchymatous cushion around the micropyle. The inner integument is only one cell thick and is generally very thin, but the cells surrounding the micropyle are elongated radially, making the layer thicker.

A globose structure of unknown function is present between the micropyle and funicle (Fig. 4a). It seems to be some kind of aril, but further discussion is precluded at present because so little is known about the ovuliferous structures of comparable living angiosperms. No palynomorphs were recovered.

Secondary xylem is well developed at the base of the peduncle, and mucilage cells surrounded by epithelial cells are abundant in the pith. Distal to the peduncle the vascular bundles subdivide to form a eustele; large oil cells are distributed in the cortex of the peduncle, each containing a globose, yellowish-brown, transparent body. Such cells also occur in the foliicle walls.

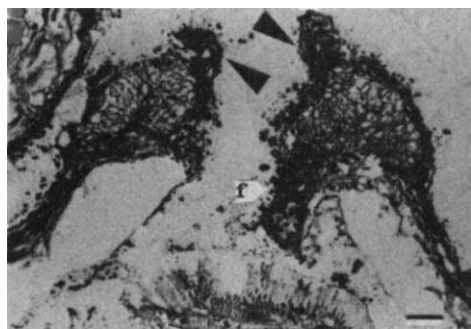


Fig. 3 Cross-section of adaxial opening of a follicle. Arrows indicate simple trichomes. Note the short funicle (f) extending from the marginal rib. Scale bar, 50 μ m.

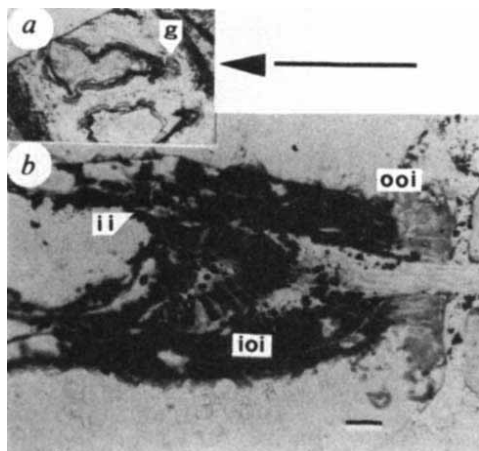


Fig. 4 Ovule structure. *a*, Median longitudinal section of two ovules. Note the small globose structure (g) attached to the micropylar end of each ovule. Scale bar, 0.2 cm. *b*, Details of the micropylar end of an ovule. ooi, Outer layer of outer integument; ioi, inner layer of outer integument; ii, inner integument. Scale bar, 50 μ m.

Based on the polycarpelous floral structure of numerous conuplicate carpels and the relatively primitive wood structure composed of mainly scalariform vessels with scalariform perforation plates, the fossil is assigned to the Magnoliales. Monimiaceae and Austrobaileyaceae are the most closely comparable families, having similar concave receptacles with apocarpous carpels. However, the carpel of these families is more specialized in lacking a prominent stigmatic crest. Carpels of Monimiaceae have a single ovule, whereas those of Austrobaileyaceae have 8–14 (ref. 12). Because the present fossil has many primitive characteristics, it is difficult to assign it to a modern family.

The carpel, with a long stigmatic crest and a large number of ovules, is unknown in any living magnolialean, but the compressed flower *Archaeanthus* Dilcher and Crane^{7,10} from the mid-Cretaceous of Kansas is comparable in this feature and also in having oil cells in the carpel walls. *Lesqueria* Crane and Dilcher and other unnamed fruits also from mid-Cretaceous of Kansas and Texas¹¹ have comparable follicle structures. The fossils differ, however, in the shape of the receptacles. All the above flowers and fruits other than the unnamed one have either elongate or ovoid receptacles on which the follicles are helically arranged, more like a living *Magnolia* flower. Note that in the early stages of magnolialean evolution, flowers evidently existed that had two quite different forms of receptacles, both of which bore carpels possessing similarly primitive features.

Although the fossil record of the early angiosperms is limited, it demonstrates through time and space the characteristics that

have existed in the evolutionary history of angiosperms. More detailed knowledge of the Cretaceous diversification of angiosperms requires further comparative studies of both fossil and living angiosperm flowers. For the present specimen to be compared with flowers or fruits of living Magnoliales, further knowledge is needed about the ontogeny and anatomy of carpels and receptacles, ovule structure, carpel arrangement and wood anatomy of living members. The use of cladistic analysis may provide a more objective evaluation of such comparative data^{3,13,14}. This method may be one of the more effective ways of answering such basic questions as what the angiosperms are, when and from what kind of ancestors they may have originated, and how they have diversified.

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Abolition of specific immune response defect by immunization with dendritic cells

Claire J. P. Boog, W. Martin Kast,
H. Th. Marc Timmers, Jolande Boes,
Leo P. de Waal & Cornelis J. M. Melief

Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, incorporating the Laboratory of Experimental and Clinical Immunology of the University of Amsterdam, PO Box 9406, 1006 AK, Amsterdam, The Netherlands

Murine cytotoxic T (T_c)-cell responses to various antigens are controlled by immune response (*Ir*) genes mapping in the major histocompatibility complex (*H-2*). The genes responsible are those encoding the class I and class II *H-2* antigens^{1–4}. The *H-2 I-A^b* mutant mouse strain bm12 differs from its strain of origin, C57BL/6 (*H-2^b*), only in three amino acids in the *I-A^b*^{bm12} class II *H-2* molecule^{5–7}. As a consequence, female bm12 mice are T_c -cell nonresponders to the male antigen H-Y and do not reject H-Y disparate skin grafts⁸. We now report that bm12 mice generate strong H-Y-specific T_c cells following priming *in vivo* and restimulation *in vitro* with male bm12 dendritic cells (DC). Female bm12 mice primed with male DC also reject male skin grafts. Furthermore, we demonstrate that only responder cell populations containing a mixture of L3T4⁺ (T-helper (T_h)) phenotype) and Lyt 2⁺ (T_c phenotype) T lymphocytes generate H-Y-specific T_c cells. These data imply an essential role for T_h cells, activated by DC as antigen-presenting cells (APC), in changing H-Y-nonresponder bm12 mice into H-Y responders. Priming and restimulation with DC allows the triggering of a T-cell repertoire not demonstrable by the usual modes of immunization. This principle might be used to overcome other specific immune response defects.

Effective T_c -cell responses to at least some antigens are elicited only in mice possessing 'responder' alleles at both class I and

class II *H-2* loci. Thus, C57BL/6 (B6) mice generate a strong H-2D^b-restricted T_c-cell response to H-Y by virtue of the presence of the *I-A^b* and *D^b* 'responder' alleles^{2,3}, illustrating effective T_h and T_c precursor activation pathways, respectively.

Previous work has demonstrated that nonresponsiveness of bm12 mice to H-Y is caused by a defect in the T_h repertoire³. Recently, dendritic cells were shown to be extremely effective in presenting antigen to T_h lymphocytes⁹⁻¹³. We therefore investigated whether it was possible to overcome the specific inability of bm12 mice to respond to H-Y by using dendritic cells as APC. To this end we used male DC to immunize female bm12 mice, and to restimulate their spleen cells *in vitro*. The ability of DC to activate H-Y-specific T_c-cell precursors was compared with that of bm12 normal spleen cells (NSC) and lipopolysaccharide (LPS)-stimulated bm12 lymphoblasts. Spleen cells from bm12 female mice primed by intraperitoneal (i.p.) injection of 10⁷ syngeneic male NSC failed to mount an H-Y-specific T_c response, irrespective of the type of cell used for *in vitro* restimulation (Fig. 1); even addition of the growth factor interleukin-2 (IL-2) during the *in vitro* restimulation did not result in the generation of H-Y-specific T_c cells. In agreement with previous results^{3,8}, all other tested immunization procedures including intravenous (i.v.) and subcutaneous footpad immunization¹⁴ with NSC failed to yield H-Y-specific T_c responses (data not shown). After priming *in vivo*, followed by restimulation *in vitro*, with male bm12 LPS blasts, a very weak H-Y-specific T_c activity was elicited. Strikingly, however, bm12 spleen cells generated strong H-Y-specific T_c activity when syngeneic male DC were used both for priming and for *in vitro* restimulation. Priming with DC followed by restimulation with NSC yielded negative results. In this case, however, addition of IL-2 resulted in an H-Y-specific T_c response (Fig. 1).

The biological significance of these findings was evaluated further using skin grafting experiments. Unprimed B6 mice or B6 mice primed with B6 male NSC all rejected male skin grafts with mean survival times of ± 23 days (Table 1). Male skin grafts on unprimed bm12 females or bm12 females primed with syngeneic NSC showed mean survival times of >120 days. McKenzie *et al.*¹⁵ showed that female bm12 mice were unable to reject male skin grafts unless they had received prior footpad priming with male NSC; the rejection rate was significantly slower than in other primed strains. As stated previously, we were unable to demonstrate H-Y-specific T_c responses in bm12 mice after footpad immunization³. In contrast, priming of bm12 females with male DC led to a dramatic decrease of male graft survival (mean time for graft rejection = 22 days; Table 1). In fact, bm12 mice primed with DC rejected their skin grafts as quickly as B6 mice primed with NSC (group 2; Table 1). The difference in survival time between the DC-primed bm12 mice and the control group was highly significant ($P < 0.001$; compare groups 6 and 4 in Table 1). All graft rejections were H-Y-specific, because all animals were also grafted with female grafts, which all survived indefinitely (>120 days). The important *in vivo* role of DC in graft rejection has also been demonstrated in allograft models^{17,18}.

Further experiments indicated the necessity for H-Y antigen to be present on the DC used for priming, because immunization

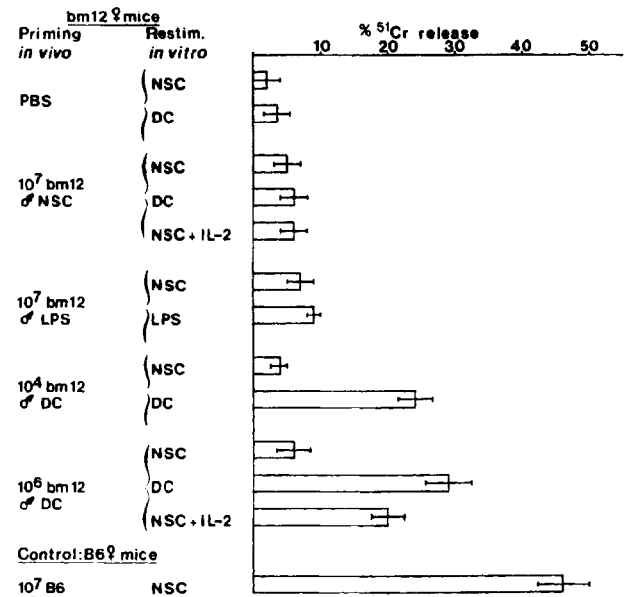


Fig. 1 H-Y-specific T_c response of bm12 *I-A* mutant mice following various modes of immunization.

Methods. Dendritic cells were isolated as described by Steinman *et al.*¹⁶. Briefly, spleen cells were spun on a discontinuous bovine serum albumin (BSA) gradient of 10, 28 and 35% BSA ($\rho = 1.099$, 1.080 and 1.031) for 30 min at 10,000g. The interphase (α band) 10–28% was removed and cultured for 90 min in glass Petri dishes. Non-adherent (Nad) cells were discarded and the medium was replaced. After a further 18 h in culture, Nad cells were separated by Fc receptor (FcR) rosetting. FcR-positive and FcR-negative (dendritic) cells were then separated over a discontinuous 10–31% BSA gradient. Fractionation of NSC yields a recovery of 9–12% α -band cells, 0.3–0.5% 18-h Nad cells and finally 0.1–0.2% FcR-negative dendritic cells. H-Y-specific T_c cells were generated as described previously³ with minor modifications. Briefly, spleen cells (10⁷) from *in vivo*-primed bm12 female mice were stimulated with irradiated (2,500 rad) male spleen cells (10⁷), LPS blasts (10⁶) or DC (10⁵) in 2 ml medium for 5 days at 37°C in humidified air with 5% CO₂. Effector cells were tested on ⁵¹Cr-labelled male and female bm12 LPS target cells (15 × 10³) at various effector/target (E/T) ratios. The results shown are those obtained at E/T = 16 (mean of five experiments, s.e.m. indicated by bars). The % lysis on female bm12 LPS target cells was always $<6\%$. The supernatant of concanavalin A-stimulated rat spleen cells (final concentration 25% in culture medium) was used as a source of IL-2.

with female DC admixed with male NSC was ineffective (data not shown). From the results shown in Fig. 1 we conclude that only after priming of bm12 female mice *in vivo* with bm12 male DC are H-Y-specific T_h cells activated; the latter are needed for the generation of sufficient numbers of T_c memory cells. This is reflected in the finding that only after priming *in vivo* with male DC can the requirement for T_h cells *in vitro* be circumvented by the addition of IL-2 (Fig. 1).

Table 1 Effect of priming of bm12 female mice with male dendritic cells (DC) on the rejection of H-Y disparate skin grafts

Group	Mice	Immunization	Grafted with	No.	MST $\pm$ s.d. (days)	P
1	B6 ♀	PBS	B6 ♂	14	22.5 $\pm$ 6	
2	B6 ♀	B6 ♂ NSC	B6 ♂	8	22.6 $\pm$ 4.0	0.10 (NS)
3	B6 ♀	B6 ♂ LPS	B6 ♂	11	11.2 $\pm$ 4.7	0.05
4	bm12 ♀	PBS	bm12 ♂	15	>120	
5	bm12 ♀	bm12 ♂ NSC	bm12 ♂	15	>120	>0.20 (NS)
6	bm12 ♀	bm12 ♂ DC	bm12 ♂	8	21.9 $\pm$ 2.1	<0.001

Mice were either unprimed or primed i.p. 3 weeks before skin grafting. All animals were also grafted with syngeneic female grafts which all survived indefinitely (>120 days). MST, mean survival time. P values were measured using the χ^2 test in comparison with phosphate-buffered saline (PBS) controls (groups 1 and 4 respectively for B6 and bm12). NS, not significant. LPS, LPS-stimulated lymphoblasts.

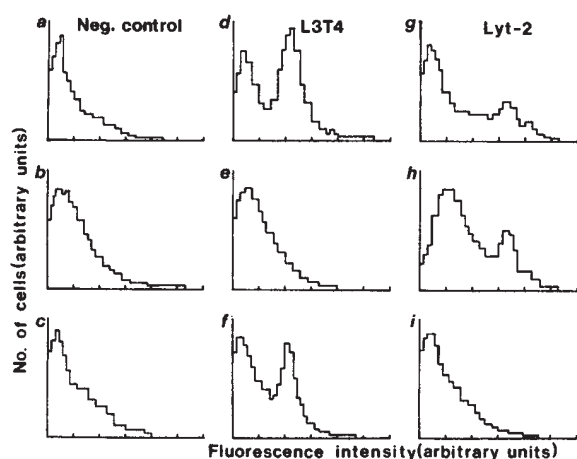


Fig. 2 Flow microfluorimetric analysis of untreated (*a, d, g*) and anti-L3T4+complement (*b, e, h*) or anti-Lyt2+complement (*c, f, i*)-treated bm12 responder cell populations (see Fig. 3 for functional analysis). Linear fluorescence histograms are expressed in arbitrary units.

Methods. After passage through nylon wool, bm12 female responder spleen cells ($2 \times 10^7 \text{ ml}^{-1}$) were incubated in Iscove's medium, 0.5% BSA, with anti-Lyt 2.2 (a mouse monoclonal antibody, 1:1,000 dilution of ascites fluid; NEN) or anti-L3T4 (a rat monoclonal antibody, 1:40 dilution of hybridoma culture supernatant SN 172-4; given by Dr H. R. MacDonald). After incubation for 45 min at room temperature, low-tox-M rabbit complement (Cedarlane) was added to a 1:10 final dilution. After incubation for 45 min at 37°C , the cells were washed and the complement treatment was repeated. Treated cells were passed over lympholyte-M (Cedarlane; density = 1.0875). Viability was $>90\%$ and cell recovery after passage through nylon wool and treatment with L3T4 or Lyt 2 + C' was $2 \pm 0.7\%$ and $3.1 \pm 0.9\%$, respectively (mean $\pm$ s.d. of five experiments). For flow microfluorimetric analysis, aliquots of 5×10^5 untreated L3T4-negative or Lyt 2-negative responder cells in $100 \mu\text{l}$ were incubated at 4°C with the following rat monoclonal antibodies: *a-c*, no antibody; *d-f*, anti-L3T4 (1:50 dilution of hybridoma culture supernatant H129-19 (ref. 30), provided by M. Pierres); *g-i*, anti-Lyt 2 (1:4 dilution of hybridoma culture supernatant 53-6.7 (ref. 31), provided by J. Ledbetter). After 30 min the cells were washed and $25 \mu\text{g ml}^{-1}$ of fluorescein isothiocyanate-conjugated rabbit anti-rat immunoglobulin (KR 116F, CLB) was added for an additional 30 min at 4°C . Control samples were stained with second-step reagent only. Cells were washed three times and all samples were passed through a flow cytometer (FACS IV; Becton and Dickinson).

In further experiments we attempted to analyse whether, during *in vitro* restimulation, bm12 mutant DC stimulate L3T4 $^+$ T $_h$ cells, or whether they are possibly more efficient in stimulating Lyt 2 $^+$ precursors of H-Y-specific helper-independent cytolytic cells. Therefore, we treated responder spleen cells with either anti-L3T4 or anti-Lyt 2 monoclonal antibody plus complement (C'), then cultured these cells with male DC as stimulator cells. Flow cytometric analysis of aliquots of the different responder cell populations (Fig. 2) showed that almost no L3T4 $^+$ (Fig. 2*e*) or Lyt 2 $^+$ cells (Fig. 2*i*) were detected after treatment with antibody and complement compared with untreated cells (Fig. 2*a, d, g*). Treatment with anti-Lyt 2 plus C', as expected, resulted in complete abrogation of the H-Y-specific T $_c$ response (Fig. 3, population 4). In addition, after removal of L3T4 $^+$ T $_h$ cells from the responder cell population, no significant H-Y-specific T $_c$ response was generated (Fig. 3, population 3). These effects were due to the specific elimination of either L3T4 $^+$ or Lyt 2 $^+$ cells, as the response could be completely restored by mixing these two treated populations (Fig. 3, population 5). We conclude that bm12 male DC are more efficient in stimulating T $_h$ cells than T $_c$ precursors.

It is unknown why DC can so efficiently activate T $_h$ cells, resulting in the abolition of the H-Y-specific immune response defect in bm12 mice. A direct relationship has been postulated

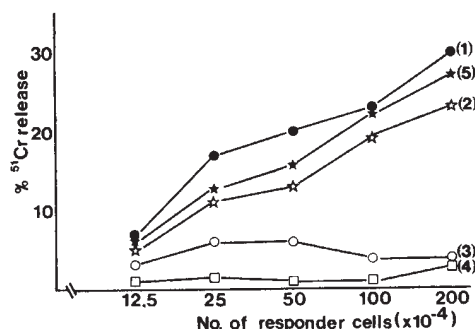


Fig. 3 Effect of responder cell treatment with anti-L3T4 or anti-Lyt 2 monoclonal antibodies plus complement on the generation of an H-Y-specific T $_c$ response of bm12 I-A mutant mice after priming and restimulation with bm12 male DC. Treatment of responder cells: population (1) untreated; (2) medium plus complement alone; (3) anti-L3T4 plus complement; (4) anti-Lyt 2 plus complement; (5) a mixture of responder cells treated with anti-L3T4 + complement (1×10^6) or anti-Lyt 2 + complement (1×10^6), respectively [populations (3) and (4)].

Methods. The different cell populations were isolated as described in Fig. 2 legend. Responder cells (2×10^6) from bm12 female mice primed *in vivo* with bm12 male DC (10^5) were cultured with irradiated (2,500 rad) bm12 male DC stimulator cells (10^5) in $200 \mu\text{l}$ of culture medium in 96-well round-bottomed microtitre plates (Flow Laboratories). After 5 days of incubation in humidified air with $5\% \text{ CO}_2$ at 37°C , various dilutions of the effector cells were tested on ^{51}Cr -labelled male and female bm12 LPS target cells (5×10^3). The % lysis on female target cells was always $<5\%$.

between APC function and the number of APC surface Ia molecules¹⁹⁻²²; our results could be in agreement with this notion as bm12 splenocytes are reported to express only 25-50% as much class II antigen as B6 splenocytes^{4,23,24}, and because DC express a large number of class II molecules^{25,26}. On the other hand, DC can be expected to overcome nonresponsiveness also in cases where no quantitative differences in class II molecules between responder (R) and nonresponder (NR) strains exist (C.J.P.B., unpublished results). In general, these effects of DC can be ascribed to (1) high expression of class II molecules and (2) qualitative properties of DC as cells specialized for the APC function. Usually, T cells from F $_1$ hybrids between R and NR strains respond only to R-type and not to NR-type APC^{27,28}; this finding neither proves nor negates defective antigen presentation by APC as the cause of nonresponsiveness (discussed by Nagy *et al.*²⁹). We previously reported, however, that (B6 $\times$ bm12)F $_1$ T cells generated an H-Y-specific T $_c$ response to either R-type B6 or NR-type bm12 male APC³, indicating that in the bm12 mouse nonresponsiveness is not caused by defective antigen presentation but by a T-cell repertoire defect³. Despite the fact that the T $_c$ response defect of bm12 mice can be overcome by immunization with DC, the repertoire defect for stimulation by NSC is still present: after priming with DC and restimulation with NSC no H-Y-specific T $_c$ response occurs, unless IL-2 is added to the culture medium. Indeed, the H-Y-specific T-cell repertoire elicited by the DC immunizations is a clearly different one, because it requires not only priming but also restimulation with male DC. This DC-unique repertoire probably reflects the presence of T $_h$ cells capable of responding to high-density I-A molecules and H-Y antigen on the surface of DC. The arousal of this dormant repertoire by immunization with DC has clear implications for states of autoimmunity, immunodeficiency and transplantation immunity.

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Phosphatidylinositol is the membrane-anchoring domain of the Thy-1 glycoprotein

Martin G. Low & Paul W. Kincade

Oklahoma Medical Research Foundation, 825 NE 13th Street, Oklahoma City, Oklahoma 73104, USA

Glycoproteins exposed on cell surfaces are commonly anchored in the membrane via hydrophobic peptide domains which penetrate the lipid bilayer¹. However, it has recently been appreciated that there are exceptions to this generalization and certain cell-surface proteins appear to be anchored via a specific association with phosphatidylinositol²⁻⁷. Thy-1 glycoprotein may also be attached to cell membranes by a non-protein hydrophobic domain located at the C-terminus and although the chemical nature of this moiety has not been determined, it was postulated that it might be a lipid⁸. On the other hand, amino-acid sequences predicted from nucleotide sequence analyses suggest that a C-terminal hydrophobic peptide segment not found in the purified, detergent-solubilized Thy-1 glycoprotein may be responsible for attachment⁹⁻¹¹. We report here that a highly purified phospholipase C specific for phosphatidylinositol selectively released Thy-1 from viable normal or malignant T lymphocytes. This result supports the proposed lipid nature of the Thy-1 anchoring domain⁸ and further suggests that this lipid is, or is closely related to, phosphatidylinositol.

EL-4 murine thymoma cells were incubated with phosphatidylinositol-specific phospholipase C (PI-PLC) purified from *Staphylococcus aureus* culture supernatants¹². The cells were washed in media, stained with fluorescently labelled anti-Thy-1 antibodies and analysed by flow cytometry. Treatment with PI-PLC produced substantial decreases in cell-surface Thy-1, as indicated by a downward shift in relative cell fluorescence (Fig. 1a). The reduction in Thy-1 density after PI-PLC treatment

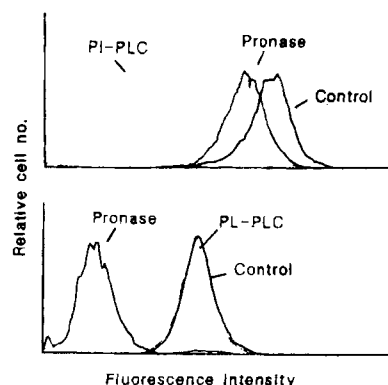


Fig. 1 Cells were incubated with PI-PLC or pronase and analysed for surface antigen as described in Table 1 legend. For clarity, the location of the unstained controls is not shown, but data from this and other experiments normalized with respect to unstained controls are shown in Table 1. a, Thy-1 antigen on EL4 cells; b, 14.8 antigen on 70Z/3 cells.

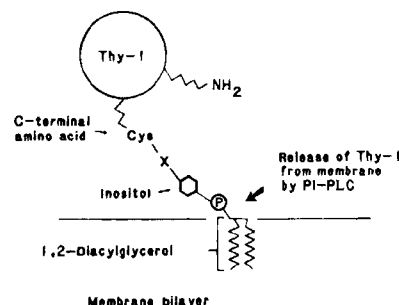


Fig. 2 A hypothetical model for Thy-1 membrane attachment. As no hydrophobic peptide domains are present in mature Thy-1 and it is released from the membrane by PI-PLC, the acyl chains of phosphatidylinositol are proposed to be entirely responsible for membrane anchoring as a result of hydrophobic interactions within the lipid bilayer. The Thy-1 polypeptide is covalently attached at the C-terminal amino-acid residue (cysteine) to the inositol ring of phosphatidylinositol via a structure (X) which at present is unidentified but appears to contain ethanolamine, glucosamine and galactosamine⁸. Similarly, the nature of the X-phosphatidylinositol linkage is unknown but probably involves one of the five free hydroxyl groups on the inositol ring.

typically amounted to a 50% reduction in logarithmically expressed fluorescence intensity and, in some experiments, a proportion of the cells was completely denuded of antigen. The effect of PI-PLC on Thy-1 was not restricted to tumour cells, as a reduction in surface Thy-1 fluorescent labelling was also observed when normal thymocytes prepared from mouse thymus were treated with PI-PLC (Table 1). By contrast, treatment of the cells with pronase (at a concentration 100 times greater than that of PI-PLC) produced a relatively small decrease in the amount of cell fluorescence (Fig. 1a, Table 1). This result indicates that Thy-1 is relatively insensitive to proteolysis, as suggested by a previous study¹³, and that loss of Thy-1 from the cell surface as a result of PI-PLC treatment is probably not due to a minor proteolytic contaminant of the highly purified PI-PLC. This view was further strengthened by the observation that other lymphocyte surface proteins such as the 14.8 antigen (on 70Z/3 cells) and the transferrin receptor (on EL4 cells) were not removed by PI-PLC even though these proteins are highly sensitive to pronase (Fig. 1b, Table 1).

The transferrin receptor has previously been shown to contain covalently bound fatty acid but it is not known whether this is involved in membrane anchoring¹⁴. It should be emphasized that PI-PLC purified by the procedure used here has previously been shown to have no detectable proteolytic activity against a

Table 1 Effect of phosphatidylinositol-specific phospholipase C on lymphocyte surface antigens

Antigen	Cells	Treatment	Fluorescence intensity (% of control)
Thy-1	EL4	PI-PLC (<i>S. aureus</i>)	51.4 ± 12.6 (7)
		Pronase	83.1 ± 10.7 (4)
		PLC (<i>B. cereus</i>)	97.6 (1)
		PLC (<i>C. perfringens</i>)	98.5 (1)
Thy-1	Mouse thymocytes	PI-PLC (<i>S. aureus</i>)	58.0 (2)
		Pronase	70.0 (1)
		PLC (<i>B. cereus</i>)	94.9 (1)
		PLC (<i>C. perfringens</i>)	96.4 (1)
Transferrin receptor	EL4	PI-PLC (<i>S. aureus</i>)	101.7 ± 31.5 (3)
		Pronase	0.8 (2)
14.8	7OZ/3	PI-PLC (<i>S. aureus</i>)	103.6 ± 4.2 (3)
		Pronase	26.9 (2)

Cells (10^6 ml^{-1}) were incubated for 60 min at 37°C in RPMI 1640/HEPES medium containing bovine serum albumin (2 mg ml^{-1}), DNase I ($200 \mu\text{g ml}^{-1}$; Calbiochem) and 2-mercaptoethanol ($5 \times 10^{-5} \text{ M}$) plus the following additions as indicated above: phosphatidylinositol-specific phospholipase C (PI-PLC), $20 \mu\text{g ml}^{-1}$ (purified from *S. aureus* culture supernatants)¹²; pronase, 2 mg ml^{-1} (*Streptomyces griseus* protease; Sigma); phospholipase C (PLC), 1 U ml^{-1} (*Bacillus cereus* grade I; Boehringer Mannheim); PLC, 5 U ml^{-1} (*Clostridium perfringens* type XII; Sigma). With the last two treatments, similar results were obtained at 10- and 100-fold lower concentrations (data not shown). The cells were centrifuged, resuspended in fresh medium and stained with fluorescently labelled antibodies. Thy-1 was demonstrated using monoclonal rat anti-mouse Thy-1.2 (clone 30-H12; Becton-Dickinson)²⁹ and monoclonal mouse anti-mouse Thy-1.2 (clone HO-13-14, from the American Type Culture Collection)³⁰; these were used as purified, directly labelled or biotin-labelled antibodies. The murine receptor for transferrin was revealed using monoclonal rat antibodies (clone R17 217) given by Dr Ian Trowbridge³¹. Monoclonal rat anti-14.8 antibody detects a macromolecule which is preferentially expressed on B-lymphocyte lineage cells³². Purified, fluorescein-labelled mouse anti-rat immunoglobulin antibodies were used as a second-step reagent as described previously³². A total of 10,000 cells per sample was evaluated with a Coulter EPICS V flow cytometer. Fluorescence was recorded as arbitrary units (channel numbers) on a logarithmic scale and median intensities determined (channel number above which 50% of cells were included). Data from individual experiments were normalized with respect to the fluorescence of unstained control samples and values are expressed as percentages ($\pm \text{s.d.}$) of the fluorescence intensities in the stained control samples. Values in parentheses are the total number of determinations in five independent experiments.

variety of substrates, to be extremely selective in the membrane components that it releases, and to hydrolyse only phosphatidylinositol and its derivatives^{5,7,15-18}. The specificity of the releasing effect of PI-PLC was further demonstrated by the observation that treatment of EL4 cells or thymocytes prepared from normal mouse thymus with two other phospholipases C which are unable to hydrolyse phosphatidylinositol^{19,20} did not have any effect on the amount of Thy-1 on the cell surface (Table 1). Thus, release of Thy-1 by PI-PLC is unlikely to be due to a physical effect resulting from perturbation of membrane structure (for example, microvesiculation) since degradation of cell-surface phospholipids using nonspecific phospholipases C would produce at least as much 1,2-diacylglycerol as the PI-PLC. The observation that the transferrin receptor and 14.8 antigen are not released (Fig. 1b, Table 1) would also argue against such a mechanism. In this regard, note that all other proteins released by PI-PLC have clearly been shown to be soluble, hydrophilic and of low relative molecular mass^{2-5,16}.

It has been suggested that the cell-surface proteins alkaline phosphatase, 5'-nucleotidase, acetylcholinesterase and the variant surface glycoprotein (VSG) of *Trypanosoma brucei*, are anchored to membranes by covalent attachment to phosphatidylinositol^{2,4-7,21}. The hydrophobic membrane-anchoring domains of the latter two proteins, which are located at the

C-terminus, have several unusual structural features. They contain ethanolamine, glucosamine and phosphatidylinositol (or inositol) but no hydrophobic amino acids^{6,7,21,22}, which suggests that the hydrophobic character and membrane attachment function in these proteins are due entirely to the acyl chains of the phosphatidylinositol. Some of these structural features of the C-terminal domain have also been observed in the Thy-1 antigen⁸. The data presented above clearly demonstrate that a substantial proportion of the Thy-1 expressed on the surface of thymocytes is selectively released by treatment with PI-PLC from *S. aureus*, and support the suggestion of Williams and co-workers⁸ that a covalently attached lipid, probably phosphatidylinositol (Fig. 2), is responsible for membrane attachment.

The role of lipid in the anchoring of Thy-1 to the membrane was recently challenged by the observation that the primary messenger RNA translation product of the *thy-1* gene, deduced from complementary DNA and genomic sequences⁹⁻¹¹ contains an extra sequence of 31 amino acids at the C-terminus which is not found in the mature protein⁸. Multiple genes, tissue/species differences, post-transcriptional mRNA processing, or post-translational protein processing do not appear to be responsible for this discrepancy^{10,11}. As this extra C-terminal sequence is extremely hydrophobic, Silver and co-workers suggested that it, instead of a lipid, is responsible for membrane attachment⁹⁻¹¹. However, the data presented here directly challenge this view as most of the surface Thy-1 on lymphocytes was released by a PI-PLC that does not cleave peptide bonds¹⁵⁻¹⁷.

Although Silver and co-workers found no evidence for post-translational proteolytic processing¹⁰, we propose that the discrepancy between the cDNA and amino-acid sequences arises as a result of the removal of the 31-amino-acid hydrophobic sequence from the C-terminus before further modification by addition of the phosphatidylinositol-containing structure. It is important to note that this same sequence of events has already been proposed for the biosynthesis and post-translational modification of the VSG of *T. brucei*^{7,23,24}. It is possible that multiple forms of Thy-1 exist and our studies, which are concerned with only those molecules exposed at the murine lymphocyte surface, do not preclude such a possibility. However, the suggestion that artefactual losses of the hydrophobic C-terminal peptide, during purification and sequence analysis, underlie the discrepancy⁹⁻¹¹ seems unlikely.

These findings beg the question of the function of this unique form of membrane attachment for particular cell-surface proteins. In the case of the VSG of trypanosomes, a specific endogenous phospholipase C has been found⁶ and it is believed that this may be important for loss of surface antigen during differentiation^{23,24}. Thy-1 is a cell-surface glycoprotein which has been well characterized with respect to distribution in animals and man, appearance in ontogeny, and amino-acid sequence²⁵. Although the function of Thy-1 is unknown, it is thought to be a member of an immunoglobulin-like family of cell-surface molecules which are involved in recognition processes^{25,26}. It is not clear whether circumstances exist in which Thy-1 is released from neuronal and lymphoid cell surfaces. However, as phosphoinositide-specific phospholipases C, which are widely distributed in mammalian tissues, are believed to be activated during cell stimulation²⁷, this possibility should be considered.

Since submission of this paper, we learned of more detailed analyses of the hydrophobic C-terminal domain of Thy-1 (ref. 28) which reveal the presence of inositol, phosphate, glycerol and fatty acid. This result strongly supports the argument presented here that phosphatidylinositol is the membrane-anchoring domain of Thy-1.

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Thrombospondin binds falciparum malaria parasitized erythrocytes and may mediate cytoadherence

David D. Roberts*, James A. Sherwood†, Steven L. Spitalnik*, Lindsey J. Pantong†, Russell J. Howard†, Vishva M. Dixit†, William A. Frazier‡, Louis H. Miller† & Victor Ginsburg*

* Laboratory of Structural Biology, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, and
† Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205, USA

‡ Department of Biological Chemistry, Division of Laboratory Medicine, Washington University School of Medicine, St Louis, Missouri 63110, USA

Plasmodium falciparum infected erythrocytes containing mature trophozoites and schizonts sequester along venular endothelium¹ and are not in the peripheral circulation of patients with malaria. Knobs appear on infected erythrocytes and are the points of attachment to endothelium². Sequestration may protect the parasite from splenic destruction³ and may play a role in the pathogenesis of cerebral malaria⁴. Correlates of sequestration have been developed *in vitro* using cultured human endothelium⁵ and an amelanotic melanoma cell line⁶. Knobless strains (K-) of *P. falciparum* fail to sequester *in vivo* and to bind to cells *in vitro*. We now present evidence that the receptor for cytoadherence is the glycoprotein, thrombospondin. *Aotus* monkey or human erythrocytes containing knobby (K+) but not *Aotus* erythrocytes containing knobless strains of *P. falciparum* bind to immobilized thrombospondin. Neither binds to the adhesive proteins laminin, fibronectin, factor VIII/von Willebrand factor or vitronectin. Both soluble thrombospondin and anti-thrombospondin antibodies inhibit binding of parasitized *Aotus* erythrocytes to immobilize thrombospondin and to melanoma cells which secrete thrombospondin.

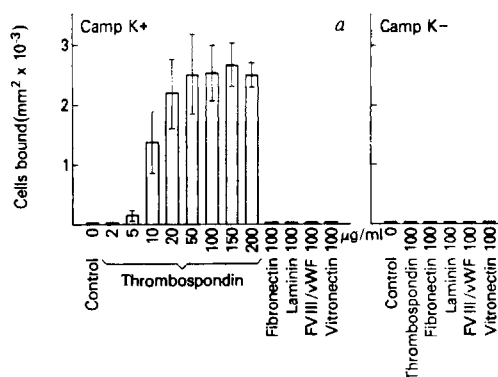


Fig. 1 Binding of *P. falciparum* parasitized erythrocytes to adhesive proteins adsorbed on plastic. **a**, K+ strain; **b**, K- strain.

Methods. Knobby (K+) and knobless (K-) variants of Malayan Camp strain *P. falciparum*²² were maintained in Columbian night monkeys (*Aotus trivirgatus griseimembra*)²⁶. Blood with 20-40% parasitaemia was cryopreserved as rings²⁷. The rings were thawed, cultured for 20-30 h to mature trophozoites and schizonts^{25,28}, washed twice in RPMI 1640, and resuspended to a 1.5-2.0% haematocrit in RPMI 1640. Viable platelets and leukocytes do not survive the cryopreservation and were not seen in the cultures. Calcium-replete human platelet TSP (isolated as described in ref. 20), human plasma fibronectin (Collaborative Research, Inc.), mouse laminin¹⁰, human factor VIII/von Willebrand factor (FVIII/vWF)²⁹, and human plasma vitronectin¹¹ in phosphate-buffered saline pH 7.4, containing 1 mM CaCl₂, were adsorbed onto bacteriological plastic dishes (Falcon 1007) by incubation in a humidified atmosphere for 3 h at 25 °C or overnight at 4 °C. These conditions result in efficient adsorption of the proteins based on immunoreactivity of adsorbed TSP¹⁴ and promotion of cell adhesion by adsorbed fibronectin, laminin and vitronectin^{9,11,30}. Control disks were incubated with phosphate-buffered saline alone. A circular area 3 mm in diameter coated with protein could be reproducibly obtained using a 10-µl drop of protein solution. After incubation, the protein solution was aspirated and the plate was immediately immersed into 50 mM Tris-buffered saline pH 7.8, containing 1% bovine serum albumin, 5 mM CaCl₂ and 0.1 mM phenylmethylsulphonyl fluoride to minimize nonspecific binding. After 30-60 min at 25 °C, the plate was rinsed in RPMI 1640 and overlaid with a suspension of parasitized erythrocytes. The plates were incubated at 37 °C for 60 min with gentle agitation every 15 min, washed by dipping in RPMI 1640, fixed for 2 h in phosphate-buffered saline, pH 7.4, containing 1% glutaraldehyde, and stained with 1% Giemsa. Results are presented as the mean $\pm$ 1 s.d. ($n = 4$).

Several adhesive glycoproteins are associated with endothelial cells or are present in blood, including thrombospondin (thrombin-sensitive protein⁷, glycoprotein G⁸, TSP), fibronectin⁹, laminin¹⁰, vitronectin¹¹ and factor VIII/von Willebrand factor¹². Adherence of Malayan Camp strain K+ *P. falciparum* parasitized *Aotus* erythrocytes to plastic surfaces coated with each of these glycoproteins or with serum albumin was tested (Fig. 1). Parasitized erythrocytes adhered avidly to surfaces coated with TSP but not to surfaces coated with laminin, fibronectin, factor VIII/von Willebrand factor, vitronectin or serum albumin. Adherence to TSP was detectable using 5 µg ml⁻¹ TSP for coating and was maximal at 50 µg ml⁻¹ TSP. Of the cells bound to TSP, 85-95% contained parasites (Fig. 2), whereas the parasitaemia of the erythrocytes used in this assay was only 10%. Uninfected erythrocytes cultured under the same conditions used for the parasitized cells did not bind to any of the proteins tested. Binding to TSP is specific for erythrocytes containing mature trophozoites or schizonts. Ring forms, the immature parasites within erythrocytes, do not sequester *in vivo* and did not bind to TSP.

A Camp K- strain of *P. falciparum*³ was also examined for adherence to proteins adsorbed on plastic (Fig. 1b). K- parasitized *Aotus* erythrocytes did not bind to TSP or the other adsorbed proteins. The correlation between knobby phenotype

Table 1 Binding of erythrocytes parasitized with different strains of *Plasmodium falciparum* to thrombospondin and to C32 melanoma cells

Parasite strain	No. of parasitized erythrocytes bound:	
	Per mm ² of thrombospondin-coated plate*	Per 100 melanoma cells†
Aotus erythrocytes		
Camp K+	7,100 ± 240	1,960 ± 90
Camp K-	25 ± 32	5 ± 2
St Lucia K+	4,100 ± 600	1,040 ± 50
St Lucia K-	30 ± 27	7 ± 3
Human erythrocytes		
Brazil ItG2F6	600 ± 350	170 ± 90
Vietnam/Cambodia (V1)	530 ± 350	290 ± 80
Thailand (T2)	220 ± 160	70 ± 30
Kenya (K3)	210 ± 140	35 ± 10
Liberia (L)	100 ± 90	115 ± 90
Nigeria (N1)	40 ± 60	50 ± 50

Parasites of the Malayan Camp²² and St Lucia²³ strains were maintained in *Aotus* monkeys and cultured synchronously to 6–20% total parasitaemia by the methods described in Fig. 1 legend. K+ and K- strains were maintained in non-splenectomized and splenectomized monkeys, respectively³. Parasites of the Brazil (ItG2F6 (ref. 24), a clone by limit dilution of It), Vietnam/Cambodia (V1), Thailand (T2), Kenya (K3), Liberia (L) and Nigeria (N1) strains all have knobs¹³ and were cultured in O⁺ human erythrocytes with O⁺ serum²⁵, cryopreserved and thawed²⁷, and cultured asynchronously in fresh erythrocytes for 10–20 days to 2–14% total parasitaemia (0.3–6% mature trophozoites and schizonts). TSP and melanoma binding assays were done four times during this period. Binding assays were performed as in ref. 6 and in Fig. 1. Binding of unparasitized erythrocytes to TSP-coated plates was 86 ± 40 cells mm⁻².

* Mean ± s.d., n = 4.

† Mean ± s.d., n = 5.

and TSP binding was further examined using another strain of *P. falciparum* (Table 1). As was found with the Camp strain, *Aotus* erythrocytes infected with the St Lucia K+ strain, but not the St Lucia K- strain, bound avidly to TSP. Only K+ strain parasitized erythrocytes bind to melanoma⁶ and endothelial cells⁵. Thus both sequestration and binding to TSP require knobs.

Although knobs are necessary for sequestration⁶, they are not sufficient¹³. Knob-positive strains of *P. falciparum* after continuous culture *in vitro* have decreased binding to melanoma or endothelial cells¹³. The strains It, V1, T2, K3, L and N1 all have knobs¹³. We measured the binding to TSP of these K+ strains cultured in human erythrocytes (Table 1). All bound at reduced levels to both TSP and melanoma cells. The two strains that bound best to melanoma cells, ItG2F6 and V1, bound best to TSP. Thus, the knobby phenotype appears to be necessary but not sufficient for TSP adherence. These results also demonstrate that parasitized human erythrocytes bind to TSP.

Further evidence that TSP is a receptor on the melanoma surface for binding of *Aotus* parasitized erythrocytes is twofold. First, preincubation of parasitized erythrocytes with soluble thrombospondin inhibited adherence both to immobilized TSP and melanoma cells (Table 2). Using 200 µg ml⁻¹ TSP, binding to TSP was inhibited 91%, and binding to melanoma cells was inhibited 81%. In a control experiment, preincubation of parasitized erythrocytes with 200 µg ml⁻¹ laminin did not inhibit binding. Second, preincubation of the melanoma cells or TSP-coated plates with rabbit anti-TSP immunoglobulin but not non-immune rabbit immunoglobulin inhibited binding (Table 2). As was the case with TSP inhibition, the antibody was more effective in inhibiting binding to TSP than binding to melanoma cells. Similar results were obtained using the monoclonal mouse antibody to TSP, A2.5 (ref. 14) (Table 2), whereas two other mouse monoclonal anti-TSP antibodies, A6.1 and C6.7 (ref. 14), did not inhibit attachment (data not shown).

Table 2 Inhibition of parasitized erythrocyte binding to immobilized thrombospondin and melanoma cells by soluble thrombospondin and anti-thrombospondin antibodies

Inhibitor	Final concentration (µg ml ⁻¹)	% Of control binding to:	
		Immobilized thrombospondin	Melanoma cells
Soluble thrombospondin	20	ND	89
	50	65	ND
	100	18	ND
	200	9	19
Rabbit anti-thrombospondin antibody	4	105	110
	12	88	ND
	40	20	63
	125	3	32
Non-immune rabbit IgG	125	120	90
Mouse monoclonal anti-thrombospondin A2.5			
	50	15	51

Rabbit anti-TSP antibodies and mouse monoclonal anti-TSP antibody A2.5¹⁴ were purified on Affigel-protein A (Bio-Rad). To determine the effect of TSP on binding, Camp K+ strain parasitized *Aotus* erythrocytes with 10% parasitaemia were incubated at 2% haematocrit in RPMI 1640 containing different concentrations of purified TSP at 25 °C for 30 min and then placed over TSP-coated plates (100 µg ml⁻¹) or formalin-fixed C32 melanoma cells. The TSP binding assay was continued as in Fig. 1. The melanoma cytoadherence assay was performed as in ref. 6. To determine the effect of rabbit anti-TSP antibody and mouse monoclonal anti-TSP antibody 2.5 on binding, TSP-coated plates (100 µg ml⁻¹) and melanoma cells were incubated in different concentrations of antibodies in RPMI 1640 at 25 °C for 30 min. To this solution was added a volume of parasitized erythrocytes at 10% parasitaemia and 50% haematocrit such that the final haematocrit was 2%. The TSP binding and melanoma cytoadherence assays were continued as above. ND, not determined.

A solid-phase radioimmunoassay for TSP using rabbit anti-TSP immunoglobulin coated on 96-well microtitre plates in 0.1 M sodium carbonate, pH 9.6, and ¹²⁵I-TSP¹⁵ (10 µCi µg⁻¹, 50 ng ml⁻¹) was used to detect TSP synthesis by C32 melanoma cells. To remove immunoreactive bovine TSP, melanoma cells which had been grown in RPMI 1640 containing 10% fetal bovine serum were preincubated for 16 h in medium containing 10% heat-inactivated horse serum, which does not contain immunoreactive TSP. For determination of TSP secretion, fresh medium containing 10% horse serum was incubated with the cells for 24 h, centrifuged for 10 min at 1,000g and assayed by radioimmunoassay. Dilutions of TSP in 10% horse serum were used as standards. The melanoma cells secreted 13 µg TSP per 10⁶ cells per 24 h. Human umbilical cord endothelial cells also synthesize and secrete TSP¹⁶. Thus, both target cells for cytoadherence *in vitro*, the melanoma cells and human endothelial cells, produce TSP. Immunofluorescence analysis of bovine aortic endothelial cells¹⁷ suggested that TSP is localized both intracellularly and as a component of fibrillar extracellular matrix.

A trypsin-sensitive strain-specific plasmodial protein of relative molecular mass 260,000–285,000 (ref. 18) on the surface of K+ but not K- parasitized erythrocytes¹⁹ has been implicated as a ligand for cytoadherence. However, the parasite could also expose a host ligand for TSP binding. TSP agglutinates trypsinized glutaraldehyde-fixed erythrocytes²⁰ and sulphated glycoconjugates on the fixed erythrocyte probably mediate agglutination¹⁵. The sulphated polysaccharides heparin and fucoidan bind to TSP and inhibit fixed erythrocyte agglutination¹⁵. We have shown that TSP binds parasitized erythrocytes and that heparin and fucoidan do not inhibit this binding (D.D.R., unpublished results). Therefore, a different site on TSP is probably recognized by the parasite.

Although parasitized erythrocytes bind directly to TSP adsorbed onto plastic, TSP itself may not be the only molecule

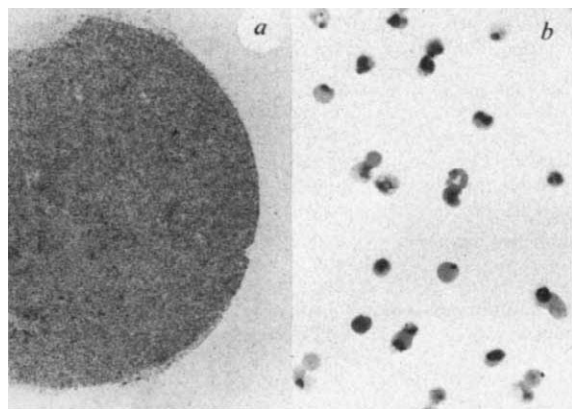


Fig. 2 Binding of *P. falciparum* parasitized erythrocytes to human platelet thrombospondin. A 100 $\mu\text{g ml}^{-1}$ solution of TSP was adsorbed onto plastic dishes, and binding of Malayan Camp K+ parasitized *Aotus* erythrocytes was determined as described in Fig. 1. The dishes were fixed in 100% methanol and stained with Giemsa. The granular material bound to a 3-mm circular area coated with TSP (a, $\times 17$) consists of stained parasitized erythrocytes viewed at high magnification in b ($\times 300$).

required for cytoadherence. Since TSP is a soluble protein, receptors for TSP are probably present on the cell membrane or extracellular matrix of melanoma cells. Thus other melanoma surface molecules may be necessary in conjunction with TSP for cytoadherence. Requirement for a TSP receptor is also suggested by the finding that some cell lines which secrete TSP in culture do not bind parasites (J.A.S., manuscript in preparation).

We have established that K+ strains of *P. falciparum* in *Aotus* erythrocytes attach specifically to immobilized TSP but not to other cell adhesion molecules. K+ strains of *P. falciparum* in fresh human erythrocytes also attach to TSP. TSP is produced by both melanoma and endothelial cells¹⁶ and may be on the surface of these cells. This evidence, combined with the blocking of cytoadherence to melanoma cells by TSP and by antibodies to TSP, is consistent with the hypothesis that TSP is a melanoma and endothelial cell receptor for *P. falciparum* cytoadherence. Binding of *P. falciparum* strains to TSP correlates well with binding to melanoma cells *in vitro*. Binding to melanoma cells correlates well with binding to endothelial cells^{6,13} and with sequestration *in vivo*^{6,21}. Sequestration occurs at specific vascular sites, including cardiac venules¹. If TSP is the receptor for cytoadherence *in vivo*, endothelium in these vessels may selectively express TSP on their luminal surfaces.

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Sequence of human tissue inhibitor of metalloproteinases and its identity to erythroid-potentiating activity

Andrew J. P. Docherty, Alan Lyons, Bryan J. Smith, Edwina M. Wright, Paul E. Stephens & Tim J. R. Harris

Division of Molecular Biology, Celltech Limited, 244-250 Bath Road, Slough SL1 4DY, UK

Gillian Murphy & John J. Reynolds

Cell Physiology Department, Strangeways Research Laboratory, Cambridge CB1 4RN, UK

Collagen fibres form the stable architecture of connective tissues and their breakdown is a key irreversible step in many pathological conditions^{1,2}. The destruction of collagen is usually initiated by proteinases, the best known of which is the metalloproteinase collagenase (EC 3.4.24)³. Collagenase and related metalloproteinases are regulated at the level of their synthesis and secretion, through the action of specific stimuli such as hormones and cytokines, and also at the level of their extracellular activity through the action of a specific inhibitor, TIMP (tissue inhibitor of metalloproteinases)⁴⁻⁶, which irreversibly forms inactive complexes with metalloproteinases⁷. Although the mechanisms governing the production of TIMP are unknown, immunologically identical forms of this glycoprotein have been detected in a wide variety of human body fluids and cell and tissue culture media^{5,8}. We therefore suggested⁵ that under physiological conditions this ubiquitous inhibitor predominates over active metalloproteinases and that tissue destruction may arise when any perturbation of this controlling excess arises. However, further progress towards testing this theory has been hindered by a lack of knowledge about the structure of TIMP and insufficient material for studying it in model systems. Here we describe the structure of TIMP predicted from its complementary DNA, its synthesis in *Escherichia coli* and transfected animal cells, and the finding that it is identical to a protein recently reported to have erythroid-potentiating activity (EPA)⁹.

TIMP was sequenced after purification from human amniotic fluid¹⁰ and from the culture media of human fetal lung fibroblasts (American Type Culture Collection CCL153). Figure 1a compares these N-terminal sequences with that published for a collagenase inhibitor¹¹, with properties similar to TIMP⁸, that is produced by human skin fibroblasts. Human amniotic fluid and human fetal lung TIMP are identical for all 28 N-terminal amino acids identified. Except for a leucine (instead of lysine at position 22) the human skin fibroblast inhibitor is also identical for 23 N-terminal residues¹¹ (Fig. 1b). Based on this information, we constructed a single 69-base oligonucleotide probe capable of encoding the 23 N-terminal amino acids of TIMP¹² (Fig. 1c). The codons used were those reported to appear most frequently in human genes, except in three positions where to

Fig. 1 N-terminal amino-acid sequences of human fetal lung fibroblast and human amniotic fluid TIMP (a) and of human skin fibroblast collagenase inhibitor (b). c, The 69-base oligonucleotide probe; d, the complementary 18-mer. The numbers above the probe sequence refer to the nucleotide sequence of the cDNA (Fig. 2b). The letters above the probe sequence represent those nucleotides that are different in the cDNA.

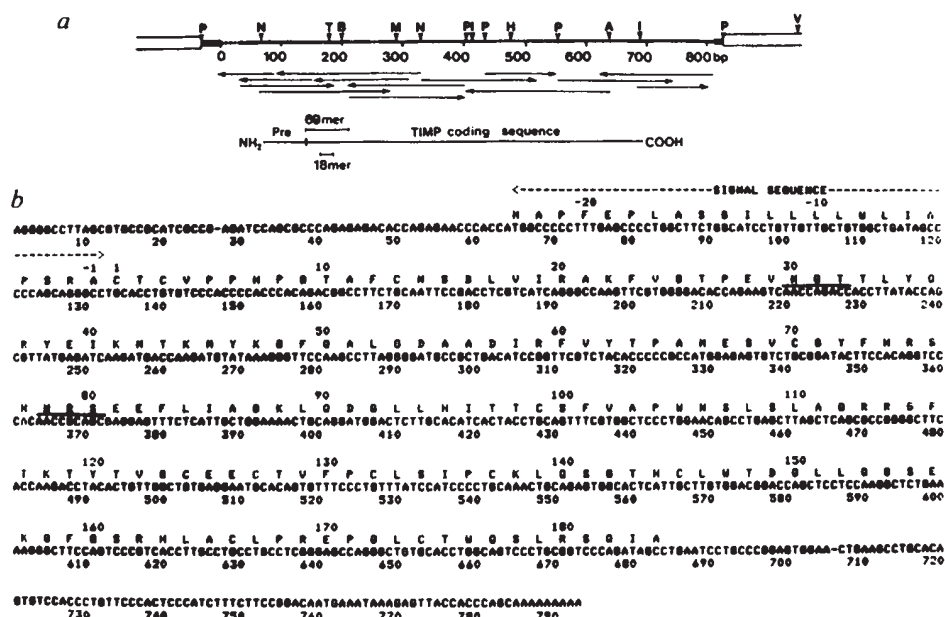
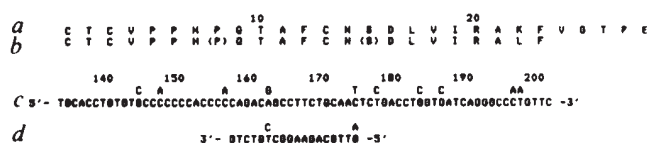
Methods. Human fetal diploid lung cells (ATCC CCL153) purchased at passage 13 (Flow Laboratories) were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with 4 mM glutamine, 15% heat-inactivated fetal calf serum (FCS) and 100 IU ml⁻¹ penicillin and 100 µg ml⁻¹ streptomycin. Cells were expanded into two 490-cm² plastic roller bottles, allowed to reach confluence in growth medium, washed with serum-free DMEM and then maintained in serum-free media for up to 6 days. Culture supernatant TIMP was measured by inhibition of rabbit skin collagenase using the collagen fibril assay¹⁰. One unit of TIMP inhibits 2 units of collagenase (2 µg of collagen degraded per min) by 50%. TIMP levels varied from <0.2 U to 5 U per 10⁵ cells per 48 h, and low-producing cells were stimulated by the addition of 5 ng ml⁻¹ 4β-phorbol-12β-myristate-13α-acetate (PMA) to the serum-free culture medium²⁴. Culture supernatants and cells were collected after 6 days, the former being concentrated 40-fold with an Amicon concentrator using a Diaflo hollow-fibre cartridge with a M_r 10,000 cut-off. TIMP was purified from the concentrated supernatants¹⁰ and reduced and carboxymethylated by standard procedures. TIMP from human amniotic fluid was similarly purified¹⁰, reduced and carboxymethylated. The NH₂-terminal sequence was determined by automated Edman degradation on an Applied Biosystems gas-phase sequencer. The 69-mer oligonucleotide probe was synthesized on an Applied Biosystems model 380A synthesizer by monomer addition of activated phosphoramidites to a solid support. The deprotected probe was purified by HPLC. The 18-mer was synthesized by automated solid-phase phosphotriester chemistry²⁵ and also purified by HPLC.

Fig. 2 a, Restriction map of human TIMP cDNA compiled from one almost full-length cDNA and the 5' end (broken line) of one additional overlapping cDNA. The open boxes represent pBR322 sequences. The arrows denote directions of dideoxy sequencing. P, *Pst*I; N, *Nco*I; T, *Tth*III-1; B, *Bst*XI; M, *Mst*II; A, *Ava*I; V, *Pvu*I; H, *Hae*II; I, *Hin*FI. b, Nucleotide sequence of human TIMP and the predicted amino-acid sequence. The numbers beneath the sequence refer to the mRNA sequence. The nucleotides at positions 27 and 706 were not identified. The numbers above the sequence refer to the amino-acid sequence; negative numbers refer to the signal sequence. Potential glycosylation sites are underlined.

Methods. mRNA was isolated from washed CCL153 cells by the guanidinium isothiocyanate/hot phenol method²⁶, followed by oligo(dT)-cellulose (Collaborative Research) column chromatography²⁷. Standard procedures were used to synthesize cDNA²⁸ and a library of 20,000 colonies was established by C-tailing the cDNA into G-tailed²⁹, *Pst*I-cut pBR322 and transforming the annealed DNA into *E. coli* DH1 (ref. 30). The colonies were transferred to nitrocellulose filters and screened with the 69-mer labelled at the 5' end to a specific activity of 2 × 10⁷ c.p.m. µg⁻¹. Hybridization was in 0.9 M NaCl, 0.09 M Tris-HCl pH 7.4, 0.006 M EDTA, 0.5% Nonidet P-40, 2× Denhardt's solution, 0.2% SDS for 16 h at 42 °C with a probe concentration of 25 ng ml⁻¹. High-stringency washing of the filters (0.5×SSC, 0.1% SDS for 15 min at 50 °C) was followed by autoradiography for 20 h with an intensifying screen. Ten putatively positive clones were identified, six of which gave positive signals after a second screening. Plasmid DNA was isolated and used as a template for T-tracking using the 18-mer as a primer^{31,32}. Five of the six clones were found to have related sequences over a region of at least 70 nucleotides and restriction mapping was used to confirm the relatedness of these clones. The complete nucleotide sequence of clone 2 (the longest clone), with some additional 5' sequence from clone 3, was determined by the dideoxy method³² using either the 18-mer to prime sequencing directly on plasmid DNA³¹ or after subcloning restriction fragments into M13 tg130 or tg131 (Amersham) and transformation into *E. coli* JM101 (ref. 33).

have included such codons would have incorporated the rarely observed dinucleotide, CpG (refs 13, 14). In addition, an 18-mer complementary to nucleotides 25–42 of the long probe was also constructed (Fig. 1d).

The 69-mer was used to identify positive colonies from a cDNA library of 20,000 clones derived from human fetal lung fibroblast messenger RNA. Restriction mapping (Fig. 2a) and partial DNA sequencing using the 18-mer as a primer revealed that five positive colonies harboured plasmids with related cDNA inserts. These were shown to be putative TIMP cDNAs by end-labelling the 18-mer and using it to prime dideoxy sequencing of the longest clone. The complete nucleotide sequence compiled from this and one other clone is shown in Fig. 2b. Examination of the sequence reveals that there is an open reading frame coding for a protein of 207 amino acids.



Evidence that the sequence encodes TIMP comes from the observation that nucleotides 133–216 encode the 28 N-terminal amino acids of human amniotic fluid and fetal lung fibroblast TIMP, including the lysine at position 22 identified by amino-acid sequencing (Fig. 1a). As the methionine encoded by nucleotides 64–66 is the only one in any reading frame upstream from the N-terminal amino acids, it is assumed that amino acids –23 to –1 (many of which are hydrophobic) represent a signal sequence that is cleaved off to liberate mature protein¹⁵. Unprocessed TIMP with the signal sequence is therefore predicted to have a relative molecular mass (*M_r*) of 23,144; this approximates to the size of TIMP core protein (*M_r* 22,000) obtained after immunoprecipitation of the *in vitro* translation products of human fetal lung fibroblast mRNA¹⁶. Furthermore, the predicted *M_r* of unglycosylated mature TIMP is 20,685, which is consistent

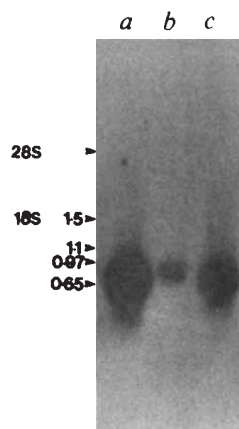


Fig. 3 Northern blot of CCL153 and HL60 mRNA probed with TIMP cDNA. *a*, CCL153 cell mRNA; *b*, mRNA from HL60 cells grown without stimulation; *c*, mRNA from HL60 cells stimulated with phorbol esters and lipopolysaccharide (LPS).

Methods. The CCL153 cells were grown as described in Fig. 1 legend. The HL60 cells were grown in a similar medium and stimulated to differentiate¹⁹ by incubation with 3×10^{-6} M PMA for 2 h, then washed and incubated for 10 h in the presence of $1 \mu\text{g ml}^{-1}$ LPS (Sigma; L-2880). mRNA was isolated as described in Fig. 2 legend and $5 \mu\text{g}$ was electrophoresed in a 1.25% agarose gel containing 8% formaldehyde, transferred to nitrocellulose and hybridized in $5 \times \text{SSC}$, $5 \times$ Denhardt's solution, 50% formamide, $300 \mu\text{g ml}^{-1}$ denatured salmon sperm DNA, 0.1% SDS, 50 mM Na_2HPO_4 pH 6.5 for 16 h at 42°C with 30 ng ml^{-1} of purified *Pst*I fragments of the cDNA shown in Fig. 2, labelled by nick-translation to a specific activity of 2×10^8 c.p.m. μg^{-1} . The filters were washed in $0.1 \times \text{SSC}$, 0.1% SDS at 60°C for 30 min and autoradiographed for 8 h. RNA sizes were estimated by running 18S and 28S RNA and ^{32}P -labelled denatured λ DNA digested with *Cla*I in parallel.

with the finding that TIMP immunoprecipitated from culture supernatants of CCL153 cells grown in the presence of tunicamycin had a M_r of 20,000 (20K)¹⁶. As tunicamycin inhibits the assembly of dolichol-linked oligosaccharides for the formation of asparagine-linked sugar chains on glycoproteins¹⁷, it is likely that most, if not all, the oligosaccharide chains on TIMP are *N*-linked. Two potential *N*-glycosylation sites are indicated in Fig. 2b.

The poly(A) tract preceded by the polyadenylation signal AATAAA at the 3' end of the cDNA indicates that the 3' end of the mRNA is represented¹⁸. Furthermore, a single band of ~800 nucleotides was observed after Northern blotting of mRNA from human fetal lung fibroblasts with TIMP cDNA, indicating that the cDNA was very close to full length (Fig. 3a). A similar-sized single band was also observed after probing mRNA from HL60 cells which had been stimulated with phorbol esters to achieve monocyte differentiation¹⁹ (Fig. 3b, c).

To confirm that the cDNA described above encodes TIMP, part of the cDNA without the signal sequence but with an additional N-terminal methionine was inserted into the *E. coli* expression vector pMG196 (E.M.W., G. O. Humphreys and G. T. Yarranton, in preparation) to produce pMG454 (Fig. 4a). In this plasmid the *trp* promoter is used to drive transcription of the inserted TIMP cDNA. pMG454 exists stably in *E. coli* at low copy number in the absence of selection but on induction increases in copy number to 100–200 per chromosome. As shown in Fig. 4c, after induction of *E. coli* cells harbouring pMG454, we observed a protein of M_r 20,000, the expected size for unglycosylated TIMP. Note that β -lactamase (which is also encoded by pMG454) was also specifically induced. By Western blotting it was shown that the 20 K protein reacts with polyclonal antibodies directed against TIMP purified from human amniotic fluid²⁰ (Fig. 4d, lane 2). As with several other recombinant proteins expressed in *E. coli*²¹, TIMP was found to be mostly in an insoluble form in inclusion bodies (data not shown).

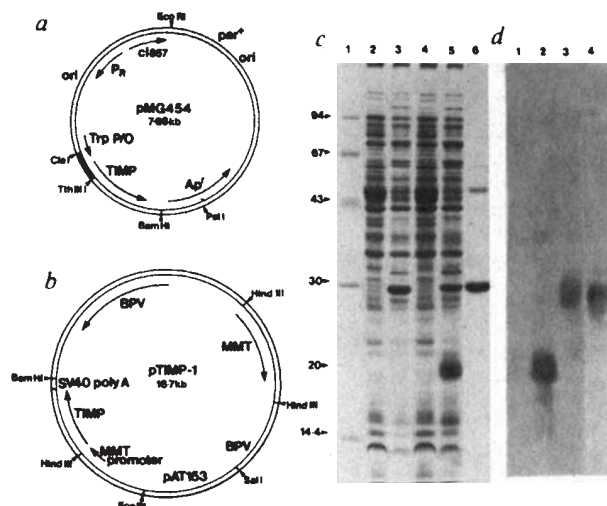


Fig. 4 *a, b*, Restriction maps (not to scale) of the *E. coli* expression vector pMG454 and the mammalian cell expression vector pTIMP-1, respectively. In pMG454 the solid box represents the 61-base pair (bp) linker. *c*, A 12% SDS-polyacrylamide gel showing Coomassie blue-stained proteins made in *E. coli* E103S transformed with pMG454. Lane 1, marker proteins; lane 2, non-induced control cells harbouring the expression vector with no TIMP cDNA insert; lane 3, induced control cells; lane 4, non-induced E103S cells harbouring pMG454; lane 5, as lane 4 but induced; lane 6, purified *E. coli* β -lactamase. *d*, Autoradiograph obtained after Western blotting with the TIMP antibody. Lanes 1 and 2, as lanes 4 and 5 in *c*; lane 3, purified human amniotic fluid TIMP; lane 4, culture supernatant proteins from a C127 cell line transfected with pTIMP-1.

Methods. For insertion into both expression vectors, the *Hin*II site adjacent to the TIMP stop codon (Fig. 2) was converted with a linker into a *Bam*HI site. For expression in *E. coli* the 5' end of the cDNA was reconstructed using a 61-bp linker to provide an upstream *Cla*I site followed by an ATG start codon preceding the cysteine codon at the N-terminus of mature TIMP. The resulting TIMP-coding fragment was ligated into the dual-origin vector pMG196 and transformed into *E. coli* E103S (a proteinase-deficient K-12 derivative). Cultures of E103S containing the resulting plasmid (pMG454) were grown at 30°C in L-broth to an A_{600} of 0.4, then shifted to 42°C by shaking at 65°C . On reaching 42°C they were transferred to a 37°C water bath and grown for a further 5 h, after which appropriately sized aliquots were taken and analysed by SDS-gel electrophoresis³⁴ and Western blotting³⁵. For expression in mammalian cells the *Nco*I site adjacent to the ATG start codon was converted with a linker into a *Hind*III site. The entire TIMP coding sequence was then inserted downstream from the *MMT* promoter in the BPV-based vector shown above (P.E.S., M. M. Bendig and C. C. Hentschel, manuscript in preparation). After transfection into C127 cells³⁶ by calcium phosphate co-precipitation³⁷, CdCl_2 (20 μM)- and ZnCl_2 (20 μM)-resistant foci were selected. Their ability to secrete TIMP was measured after 16 h of growth in serum-free media by a competitive RIA based on immobilized specific antibodies to TIMP²⁰, Western blotting³⁵, and a collagen fibril assay as described for CCL153 cells (Fig. 1).

The cDNA was also inserted into a vector designed for expression in mammalian cells (Fig. 4b). The coding sequence of pre-TIMP was inserted between the murine metallothionein (*MMT*) promoter and the simian virus 40 early transcript polyadenylation signal in a bovine papillomavirus (BPV)-based vector²². This plasmid (pTIMP-1) also has a functional *MMT* gene which confers heavy-metal resistance on mammary fibroblast tumour cells (C127 cells) harbouring it. Calcium phosphate co-precipitation was used to introduce pTIMP-1 DNA into these cells and nine resistant foci were selected for further study. Radioimmunoassays (RIAs) for TIMP were performed on culture supernatants after 16 h in serum-free medium. The polyclonal antibodies directed against purified human amniotic fluid TIMP, which are known not to cross-react with bovine or murine

TIMP²⁰, detected a 200-fold increase of TIMP in some of the pTIMP-1-transfected cell lines compared with control cells transfected with the plasmid without inserted cDNA. Highest yields of up to 1.5–2.0 $\mu\text{g ml}^{-1}$ TIMP were obtained. In addition, Western blotting indicated that the TIMP secreted by the transfected C127 cells had a M_r identical to authentic TIMP purified from human amniotic fluid, suggesting that in these cells the recombinant TIMP is correctly processed and glycosylated (Fig. 4d, lane 4). To determine whether the immunoreactive TIMP made in the C127 cells was biologically active, the culture supernatants were tested for their ability to inhibit rabbit skin collagenase using the collagen fibril assay¹⁰ (see Fig. 1 legend). No activity was detected in the supernatants of those cells shown by RIA to be making undetectable amounts of TIMP. However, 2–5 U ml^{-1} TIMP was detected in the supernatants of those cells shown in the RIA to be making in excess of 500 ng ml^{-1} TIMP, indicating that pTIMP-1 directs the synthesis of active TIMP in C127 cells. Further refinement of pTIMP-1-harboured cell lines should permit a greater yield of active TIMP.

The N-terminal sequences indicate that TIMP and the human skin fibroblast inhibitor are identical. Their overall amino-acid composition is also very similar, which is in keeping with the finding that these commonly occurring inhibitors, together with others of M_r 28,000 (28K) identified in many human tissues^{5,8}, are immunologically and functionally identical. The cDNA predicts the presence of 12 cysteine residues, which is in contrast to the larger number (24 half-cystines) reported for the skin fibroblast inhibitor¹¹ but which is consistent with the cysteine content reported for a bovine metalloproteinase inhibitor²³. Recently, Gasson *et al.*⁹ reported the N-terminal sequence of a 28K protein reported to have erythroid-potentiating activity (EPA) that was purified from medium conditioned by human T-cell lymphotropic virus type II (HTLV-II)-infected Mo T-lymphoblast cells. This N-terminal sequence and the predicted amino-acid sequence of the corresponding cDNA are identical to those reported here for TIMP. Although these data suggest that the enzyme inhibitor (TIMP) may have lymphokine activity on specific cells of the erythroid lineage, it will first be necessary to determine whether TIMP prepared by our methods has erythroid-potentiating activity. The TIMP-synthesizing *E. coli* and mammalian cell lines should also permit the purification of sufficient TIMP for a thorough study of the cellular mechanisms that normally prevent uncontrolled resorption. Furthermore, the primary structure of TIMP described here will provide a starting point for designing new compounds for controlling disease processes in which accelerated breakdown of extracellular matrices is a prominent feature.

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L-myc, a new myc-related gene amplified and expressed in human small cell lung cancer

Marion M. Nau, Burke J. Brooks, James Battey, Edward Sausville, Adi F. Gazdar, Ilan R. Kirsch, O. Wesley McBride*, Virginia Bertness, Gregory F. Hollis & John D. Minna

NCI-Navy Medical Oncology Branch and *Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health & Naval Hospital, Bethesda, Maryland 20814, USA

Altered structure and regulation of the c-myc proto-oncogene have been associated with a variety of human tumours and derivative cell lines, including Burkitt's lymphoma^{1,2}, promyelocytic leukaemia^{3,4} and small cell lung cancer (SCLC)⁵. The N-myc gene, first detected by its homology to the second exon of the c-myc gene, is amplified and/or expressed in tumours or cell lines derived from neuroblastoma^{6–8}, retinoblastoma⁹ and SCLC¹⁰. Here we describe a third myc-related gene (L-myc) cloned from SCLC DNA with homology to a small region of both the c-myc and N-myc genes. Human genomic DNA shows an *EcoRI* restriction fragment length polymorphism (RFLP) of L-myc defined by two alleles (10.0- and 6.6-kilobase (kb) *EcoRI* fragments), neither associated disproportionately with SCLC. Mouse and hamster DNAs exhibit a 12-kb *EcoRI* L-myc homologue, which indicates conservation of the gene in mammals. Gene mapping studies assign L-myc to human chromosome region 1p32, a location distinct from that of either c-myc^{1,11} or N-myc¹² but associated with cytogenetic abnormalities in certain human tumours¹³. This L-myc sequence is amplified 10–20-fold in four SCLC cell line DNAs and in one SCLC tumour specimen taken directly from a patient. Either the 10.0- or 6.6-kb allele can be amplified and in heterozygotes only one of the two alleles was amplified in any SCLC genome. SCLC cell lines with amplified L-myc sequences express L-myc-derived transcripts not seen in SCLC with amplified c-myc or N-myc genes. In addition, some SCLCs without amplification also express L-myc-related transcripts. Together, these findings suggest an enlarging role for myc-related genes in human lung cancer and provide evidence for the concept of a myc family of proto-oncogenes.

While examining genomic DNA from human SCLC cell lines for evidence of c-myc and N-myc amplification, we occasionally observed a number of additional *EcoRI* fragments, including 10.0- and 6.6-kb fragments that hybridized to either a second-exon c-myc probe¹⁴ or to the homologous region of the N-myc gene (pNb-1)⁶ (see Fig. 1a). We speculated that these *EcoRI*

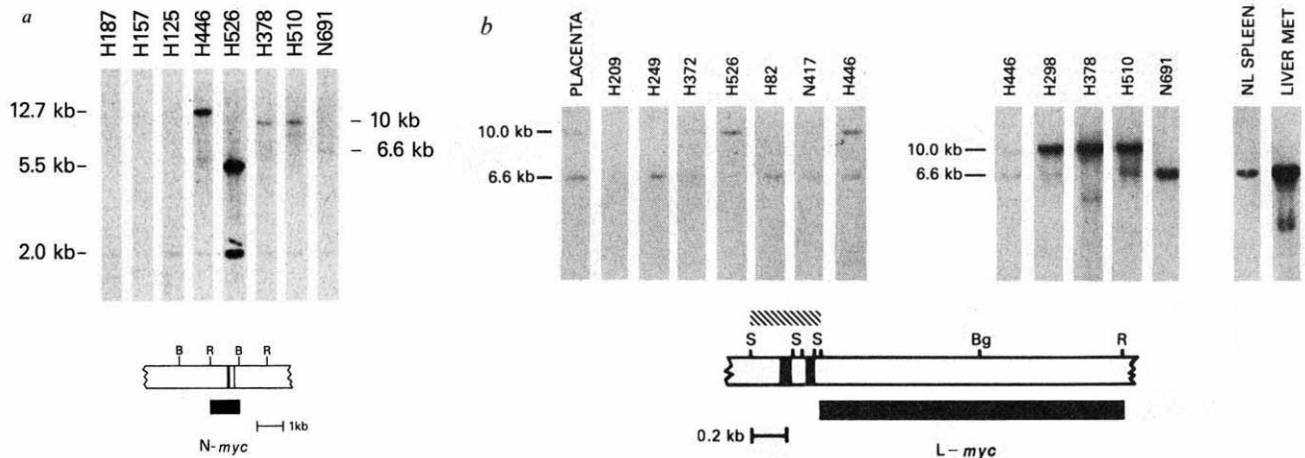


Fig. 1 *a*, Detection of novel *myc*-related sequences in *Eco*RI digests of SCLC cell line DNAs with a human *N-myc* probe. *b*, Hybridization comparison of *Eco*RI digests of SCLC cell line and patient tissue sample DNAs with a human *L-myc* probe. In *a*, the *N-myc* probe is a 1.0-kb *Eco*RI-*Bam*HI fragment (horizontal solid bar) obtained from clone pNb-1 isolated from a human neuroblastoma cell line⁶. The 12.7-kb position represents the *Eco*RI germline *c-myc* gene fragment¹ (which is seen with the *N-myc* probe when it is amplified as in H446); the 2.0-kb position, the *Eco*RI germline *N-myc* fragment⁶ seen in all DNAs in *a* and amplified in H526 (ref. 10); the 5.5-kb position, a putative *N-myc*-related gene sequence found and amplified in H526 (ref. 10); the 10.0-kb (H378 and H510) and 6.6-kb (N691) positions, the *Eco*RI *myc*-related gene fragments representing *L-myc*. In *b*, the *L-myc* probe used is the 1.8-kb *Sma*I-*Eco*RI fragment of *L-myc* was isolated by preparative gel electrophoresis²⁷ of an *Eco*RI digest of SCLC cell line H378 genomic DNA and cloned into CH4A phage²⁸. Five clones were identified using either the Nb-1 *N-myc* probe⁶ or the second-exon 1.6-kb *Sst*I-*Sst*I *c-myc* probe¹. One of these clones was subcloned into pJB327 (a derivative of pBR327)²⁹ and was termed pLmyc 10. The 10.0-kb and 6.6-kb positions represent the *Eco*RI genomic *L-myc* fragments. The degree of *L-myc* amplification was determined by cutting-out and liquid scintillation counting of the ³²P-labelled 10.0-kb and 6.6-kb *L-myc* bands from the Southern hybridization blot shown in *b*. The c.p.m. of each amplified band was divided by the average c.p.m. of a single non-amplified allele to obtain an approximate amplification value, after background c.p.m. was subtracted from both. Heavy vertical solid lines indicate regions of homology between *c-myc*, *N-myc*⁶ and *L-myc* DNA sequences; the hatched block indicates the region sequenced (see Fig. 3 legend). Restriction endonuclease sites are indicated as follows: B, *Bam*HI; R, *Eco*RI; S, *Sma*I; and Bg, *Bgl*II.

Methods. The NCI-series of cell lines that were established, characterized and grown in our laboratory^{22,23} include SCLC lines H82, H187, H209, H249, H298, H372, H378, N417, H446, H510, H526 and N691, and non-SCLC lines H125 and H157. Cell line DNA was prepared as described elsewhere²⁴, while tumour and normal tissue samples collected directly from the patient at autopsy were first pulverized in liquid nitrogen in a blender before DNA extraction. Then 12 µg of each DNA was digested with *Eco*RI, electrophoresed on a 0.8% agarose gel, denatured and transferred to nitrocellulose essentially as described by Southern²⁵. Hybridization was performed using 10% dextran sulphate²⁶ with the ³²P-labelled probe indicated on the schematic map and washed at 52 °C in 0.1 × SSC, 0.1% SDS as described previously¹.

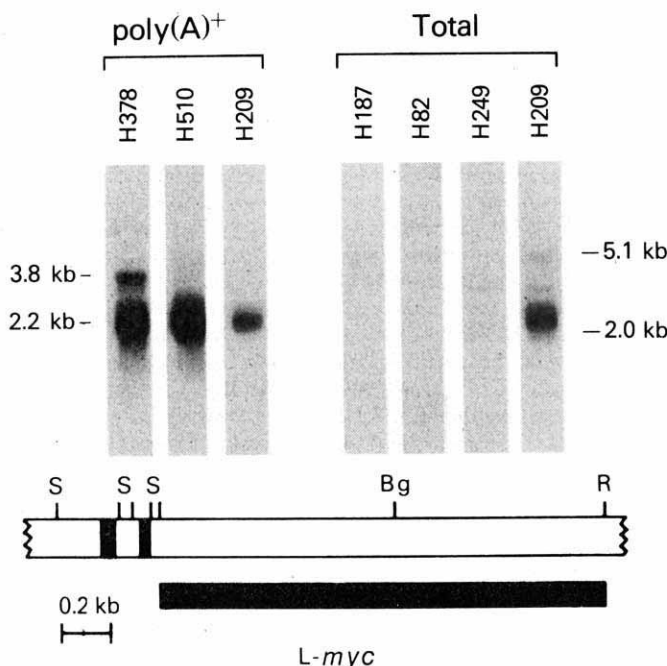


Fig. 2 Hybridization comparison of poly(A)⁺ and total SCLC cell line RNAs with a human *L-myc* probe. The 5.1-kb and 2.0-kb positions indicate 28S and 18S human ribosomal RNAs respectively.

Methods. Total cellular RNA was prepared by guanidine thiocyanate-caesium chloride gradient centrifugation³⁰. Several RNAs were further enriched by oligo(dT)-cellulose chromatography³¹. Then 10 µg of total RNA or 2 µg of poly(A)⁺ RNA was denatured and electrophoresed on a 1% agarose-formaldehyde gel³², modified by using 0.22 M formaldehyde in the gel and electrophoresing at 160 V for 4 h. Transfer, hybridization and washing were as described elsewhere³³.

myc-hybridizing fragments might represent other *myc*-related genes which were also amplified in some SCLC cell line DNAs.

To test this hypothesis, the novel 10.0-kb *Eco*RI DNA genomic fragment from human SCLC cell line NCI-H378 was cloned from *Eco*RI size-fractionated DNA and designated pLmyc 10 (see Fig. 1b legend). The partial pLmyc 10 restriction endonuclease map obtained (Fig. 1b) is markedly different from those of *c-myc*¹⁴, *N-myc*⁶ and three other *myc*-related clones described earlier¹⁵. Using restriction endonucleases, various subfragments of pLmyc 10 were subcloned, including the 1.8-kb *Sma*I-*Eco*RI fragment (see map, Fig. 1b). We have termed this new *myc*-related sequence *L-myc* because it was first isolated from a human lung cancer cell line.

Using the *Sma*I-*Eco*RI fragment as a probe, we then screened *Eco*RI digests of 40 SCLC cell line DNAs for *L-myc* sequences using Southern blot analysis (see Fig. 1b for examples). In addition to the expected 10.0-kb *Eco*RI DNA fragment, we also observed hybridization to the 6.6-kb *Eco*RI fragment in several DNAs (including human placental DNA). DNAs were identified that contained either both *Eco*RI fragments (10.0 and 6.6 kb) or only one fragment (10.0 or 6.6 kb). Since a number of other restriction endonuclease digests (*Bgl*II, *Sst*I, *Bam*HI and *Hind*III) show no pattern differences between DNAs (data not shown), these two *Eco*RI fragments identified represent an *Eco*RI restriction site polymorphism outside the *Sma*I-*Eco*RI *L-myc* probe, rather than a tumour-specific somatic rearrangement. The presence of the two alleles, however, raises the question of an association between lung cancer and the alleles, as was recently suggested for the *ras* oncogene family¹⁶. We scored 40 SCLC DNAs, 30 cell line DNAs of other tumour types including adenocarcinoma of the lung, neuroblastoma and retinoblastoma, as well as the lymphocyte DNAs of 13 normal individuals. There were no major differences in the *L-myc* allele frequencies among the three groups. Overall, 24 DNAs were 10.0-kb homozygotes, 22 DNAs were 6.6-kb homozygotes and 37 were heterozygotes. Pooling data from all tumour cell lines

Fig. 3 Nucleotide and predicted amino-acid comparison of *c-myc*, *N-myc* and *L-myc* homologous regions. The schematic map represents the human *c-myc* germline gene¹⁴ where hatched blocks indicate the first, second and third exons of the gene and heavy vertical lines the region of homology. At both the nucleotide and amino-acid levels the sequence of the *c-myc* homology region is shown, whereas only differences from *c-myc* are indicated in the *N-myc* and *L-myc* sequences. The numbers between the two homology regions represent nucleotides and amino acids separating these regions respectively. pLmyc10 was digested with appropriate restriction endonucleases and cloned into mp9, mp10 and mp11, to obtain overlapping clones and sequences of both strands. A hatched block in Fig. 1b indicates the region of pLmyc10 sequenced. The nucleotides as described elsewhere³⁵. Certain sequences were

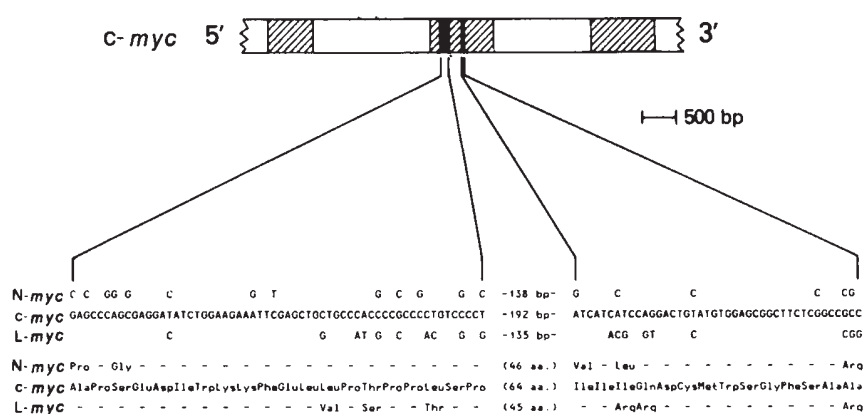
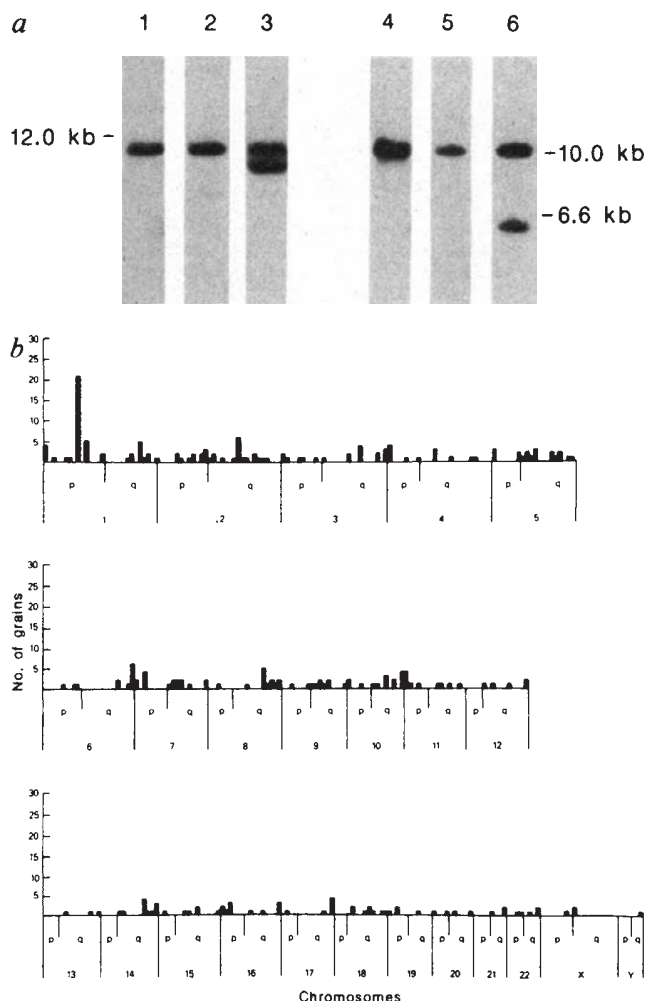


Fig. 4 *a*, L-*myc* hybridization comparison of hamster and mouse DNAs with representative human-hamster and human-mouse somatic cell hybrid DNAs. The DNA samples are as follows: lane 1, hamster control; lane 2, hamster-human hybrid negative for human L-*myc* sequences; lane 3, hamster-human hybrid positive for the 10.0-kb *EcoRI* human L-*myc* fragment; lane 4, mouse control; lane 5, mouse-human hybrid negative for human L-*myc* sequences; lane 6, mouse-human hybrid positive for the 6.6-kb *EcoRI* human L-*myc* fragment. *b*, Distribution of chromosome-associated grains from an analysis of 159 human metaphases by chromosome *in situ* hybridization of the L-*myc* probe to a karyotypically normal male. The analysis is based on a chromosome banding pattern averaging 400 bands per metaphase spread: 21 of the grains (7.7%) cluster at or near band 1p32 ($P < 10^{-5}$).



and normal individuals, the frequency of the 10.0-kb allele was 0.51 whereas the frequency of the 6.6-kb allele was 0.49 (166 alleles analysed).

Experiments also revealed several SCLC cell line DNAs, including H378, amplified for either the 10.0-kb or 6.6-kb *Eco*RI DNA fragment (NCI lines H298, 10-fold; H378, 20-fold; H510, 15-fold; and N691, 10-fold), when compared with other SCLC cell line DNAs (Fig. 1b). In addition, a SCLC tumour sample obtained directly from a patient at autopsy showed an approximate 15-fold amplification of L-*myc* DNA when compared with the patient's normal tissue DNA (Fig. 1b). Some DNAs amplified for either the 10.0-kb and 6.6-kb *Eco*RI L-*myc* fragments show other uncharacterized hybridizing species (H378

and the liver metastasis collected at autopsy) (Fig. 1b). In DNAs containing both *L-myc* alleles (10.0-kb and 6.6-kb *EcoRI*) only one of the two alleles was amplified. None of the SCLC lines with *L-myc* amplification has either *c-myc* or *N-myc* DNA amplification (data not shown). Similarly, none of the lines previously shown to be amplified for *c-myc* (H82, N417, H446)⁵ or *N-myc* (H249, H372, H526)¹⁰ was amplified for the *L-myc* gene (see Fig. 1a, b).

L-*myc* gene expression was examined in a group of SCLC lines, including those that showed L-*myc* amplification. All cell lines amplified for the L-*myc* sequences show a 2.2-kb transcript in poly(A)⁺-selected RNA using the 1.8-kb *Sma*I-*Eco*RI fragment as a probe (Fig. 2). It is of interest that H378 poly(A)⁺

Table 1 Segregation of the human *L-myc* gene in rodent-human hybrid cells (% discordancy)

Human chromosome	Hybrid cell type		Total (n = 65)
	Human-mouse (n = 32)	Human-hamster (n = 33)	
1	0, 3	0, 3	0, 3
2	13, 9	39, 27	26, 18
3	25	30	28
4	34	24	29
5	28	24	26
6	22, 38	33, 36	28, 37
7	34	48	42
8	41	39	40
9	19	45	32
10	25	42	34
11	31	42	37
12	31, 41	30, 33	31, 37
13	38	30	34
14	47	39	43
15	25	48	37
16	53	18, 30	35, 42
17	53	21	37
18	63	39	51
19	9	24	17
20	25	36	31
21	47	42	45
22	19	45	32
X	44	55	49

Detection of the human *L-myc* gene (10.0-kb and 6.6-kb *EcoRI* fragments) correlated with the presence or absence of various human chromosomes in 65 mouse-human and Chinese hamster-human primary hybrids and subclones. Discordancy represents either the presence of a specific human chromosome in the absence of human *L-myc* or the presence of this sequence despite the absence of the chromosome in that hybrid. The *L-myc* locus can be assigned to that chromosome with the lowest discordancy. When two numbers are given, they refer to discordancy with short-arm and long-arm markers, respectively. The human-mouse hybrids consist of 15 primary clones and 17 subclones of 5 additional hybrids¹⁷⁻¹⁹ and 12 contained the human *L-myc* sequence. The hamster-human hybrids consist of 23 primary¹⁷⁻¹⁹ and 10 subclones; 12 were positive for human *L-myc*. Thirty-four of the hybrids came from crosses segregating the 10.0-kb allele, 25 came from crosses segregating the 6.6-kb allele and for the remaining 6 the RFLP pattern of the parents was not determined. The hybrid cell line DNAs also provided additional information on the subchromosomal localization of *L-myc* on human chromosome 1. One mouse-human hybrid contained the 6.6-kb *L-myc EcoRI* allele, the genes for phosphoglucomutase-1 on 1p, *N-ras* on 1p and a 6.2-kb *EcoRI* metallothionein pseudogene on 1p, but lacked the genes for peptidase C on 1q and a 2.8-kb *EcoRI* metallothionein pseudogene¹⁷. A human-hamster hybrid contained the 10.0-kb *L-myc EcoRI* allele, the 6-phosphogluconate dehydrogenase gene on 1p, phosphoglucomutase-1, enolase-1 on 1p and the metallothionein 6.2-kb sequence, but lacked the genes for peptidase C, *N-ras* and the 2.8-kb metallothionein sequence. These results strongly suggest that both the 10.0-kb and 6.6-kb *EcoRI L-myc* alleles are located on human chromosome arm 1p and are distal to the *N-ras* gene on 1p (ref. 21).

RNA shows both a 2.2-kb and a 3.8-kb RNA transcript. The origin of the novel 3.8-kb species and its relationship to the 2.2-kb RNA are unclear. One other SCLC cell line (H209) not amplified for the *L-myc* gene sequence also expresses an *L-myc* RNA transcript of 2.2 kb (see Fig. 2). Two representative SCLC lines that are amplified and express increased amounts of either *c-* or *N-myc* mRNAs (H82 and H249 respectively) contain no detectable amounts of the 2.2- or 3.8-kb *L-myc* RNA transcript (see Fig. 2). Because *c-myc*-expressing SCLC cell lines do not express *N-myc* and vice versa¹⁰, it appears that only one of these three *myc*-related genes is expressed at detectable levels in total RNA from any individual SCLC cell line thus far examined.

Previous experiments comparing *c-myc* and *N-myc* sequences have demonstrated two short regions of homology between these two genes, localized to the second exon of the *c-myc* gene⁶. To understand better the structural relationship of the *L-myc* gene to the *c-myc* and *N-myc* genes, the region of homology in *L-myc* was mapped to restriction fragments located about 100 base pairs (bp) 5' to the 1.8-kb *SmaI-EcoRI L-myc* probe (see map, Fig. 1b) and the nucleotide sequence determined. Figure 3 shows a nucleotide and predicted amino-acid comparison of the

homologous regions of the *c-*, *N-* and *L-myc* genes. Like *N-myc*, *L-myc* contains the same two regions of homology to the *c-myc* gene separated by a stretch of nucleotides that bears no resemblance to the analogous region in *c-myc*. The 5' and 3' blocks of homology show about 80% nucleotide sequence homology with the analogous regions of either *c-* or *N-myc*. An open reading frame spans the 5' and 3' blocks of homology shown in Fig. 3, including the 135-nucleotide non-homologous stretch between these two regions. The predicted amino-acid sequences for the two homology regions of *L-myc* show overall 82% and 76% homology when compared with that of the *c-myc* or *N-myc* gene respectively.

The *c-myc* gene is assigned to human chromosome 8 (refs 1, 11) while *N-myc* is assigned to chromosome 2 (ref. 12), indicating dispersion of these two genes to different autosomes. We mapped the *L-myc* gene to a human chromosomal region. Southern blot analyses using the 1.8-kb *SmaI-EcoRI L-myc* probe revealed a 12-kb *EcoRI L-myc* hybridizing band in both hamster and mouse DNAs, indicating the presence of an *L-myc* homologue in these two species (Fig. 4a). Analysis of rodent-human hybrids segregating human chromosomes¹⁷⁻¹⁹ revealed either retention of the 10.0-kb or 6.6-kb fragment or neither of these human *EcoRI L-myc* fragments (Fig. 4a). A panel of 65 human-rodent hybrid clones representing 43 independent hybrids with 4 different human parents were scored for the presence or absence of human *L-myc* genes and this was correlated with the retention of each human chromosome (Table 1). Both the 10.0-kb and 6.6-kb *EcoRI L-myc* alleles segregated concordantly with human chromosome 1 and discordantly with the other human chromosomes, indicating that this autosome is the most likely location for *L-myc*.

The *L-myc* gene was also mapped using the technique of chromosome *in situ* hybridization. A total of 159 separate metaphases were analysed and the results plotted on a 400-band idiogram (Fig. 4b). Grains at 1p32 comprise 7.7% of the total grains and the probability of this distribution occurring as a random event is $P < 10^{-5}$. Thus, by both somatic cell hybridization and chromosome *in situ* hybridization techniques, the *L-myc* genes have been localized to the short arm of chromosome 1, very near or within band 1p32. This is approximately the same location to which a putative oncogene, *Blym*, was mapped²⁰ earlier but distal to the proto-oncogene *N-ras*²¹. It is interesting that the human melanoma kindred gene has also been provisionally localized to 1p (ref. 13). In addition, the *L-myc* gene and its associated *EcoRI* RFLP may prove to be a useful marker in analysing cytogenetic changes in human tumours involving the 1p32 region.

Given the well-defined and limited region of homology, it will be of great interest to see whether the three *myc* gene products have similar or related functions. SCLC cell lines that contain amplified *L-myc* DNA sequences also contain at least one *L-myc*-related RNA transcript. In addition, *L-myc* has been mapped to 1p32, a chromosomal location different from that of the *c-myc*^{1,11} or *N-myc*¹² genes. The fact that *L-myc* has been found amplified in a patient's tumour tissue, but not in the normal tissue, suggests that this gene may be important in the genesis or progression of SCLC.

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Sarcoma viruses carrying *ras* oncogenes induce differentiation-associated properties in a neuronal cell line

Makoto Noda^{*†}, Minoru Ko[†], Akihiko Ogura[‡],
Ding-gan Liu^{*}, Takehiko Amano[‡],
Toshiya Takanot[†] & Yoji Ikawa^{*}

^{*} Laboratory of Molecular Oncology, Institute of Physical and Chemical Research, Wako-shi, Saitama 351-01, Japan

[†] Department of Microbiology, Keio University School of Medicine, Shinjuku, Tokyo 160, Japan

[‡] Department of Neurochemistry, Mitsubishi Kasei Institute of Life Sciences, Machida-shi, Tokyo 194, Japan

The growth-promoting and/or differentiation-blocking activities of Kirsten (Ki-MSV) or Harvey murine sarcoma virus (Ha-MSV) on various types of cells *in vitro* are well documented¹⁻⁸. Here we report an unexpected effect of these viruses on a rat pheochromocytoma cell line, PC12. PC12 cells, which multiply indefinitely in growth medium, are known to respond to nerve growth factor (NGF) by cessation of cell division and expression of several properties resembling those of differentiated sympathetic neurones^{9,10}. We have found that Ki- and Ha-MSV mimic some, if not all, of the activities of NGF in PC12 cells, and there is evidence that the viral oncogenes, *v-Ki-ras* and *v-Ha-ras*, are responsible for this phenomenon. This system may be of value for studying the mechanism of action of the *v-ras* genes as well as the regulatory mechanism of growth and differentiation in neuronal cells.

In an initial attempt to 'super-transform' PC12 cells, we infected the cells with one of five mammalian retroviruses carrying different oncogenes. To our surprise, Ki- or Ha-MSV, carrying *v-Ki-ras* or *v-Ha-ras* respectively, induced marked morphological changes of the host cells within 2-3 days of infection, that is, enlargement of the cell bodies and extension of neurite-like processes (Fig. 1c, e). These changes were similar to those induced by NGF (Fig. 1b), but in this case it was more than 4 days before we observed processes of comparable length to those induced by the *ras*-containing viruses. Moloney (Mo) MSV, carrying the *v-mos* gene, seemed to induce some morphological changes in a few days after infection (Fig. 1f), but the cells subsequently became very large and granulous, and eventually

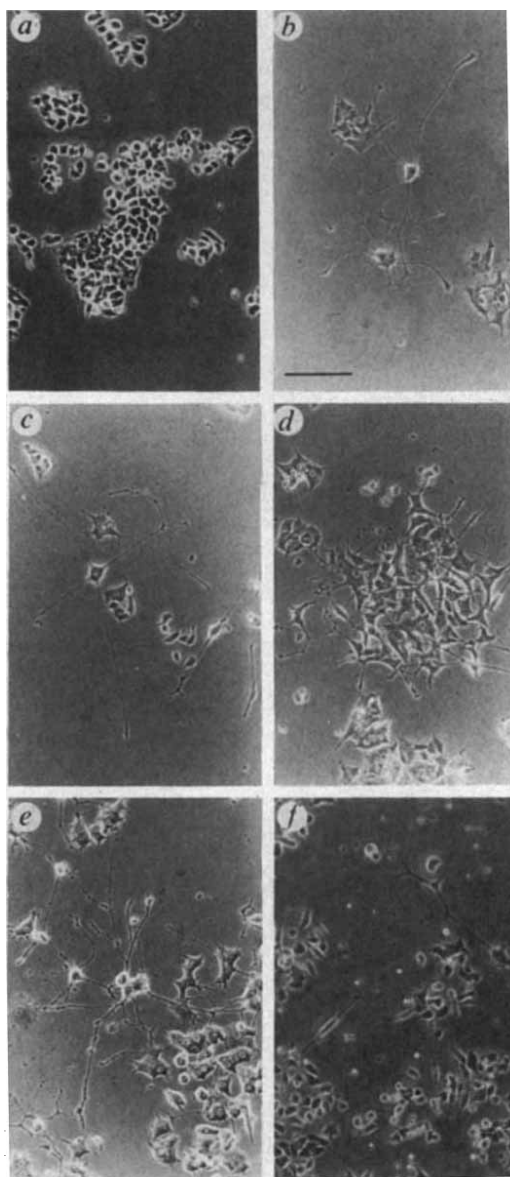


Fig. 1 Morphology of NGF-treated or MSV-infected PC12 cells. PC12 cells were usually grown at 36 °C in DMEM (Gibco) supplemented with 5% (v/v) heat-inactivated horse serum (Flow Laboratories), 5% fetal calf serum (Mitsubishi Kasei Co.), 50 U ml⁻¹ penicillin G and 50 µg ml⁻¹ streptomycin sulphate in a humidified CO₂ incubator. Virus infection was carried out in the presence of 6 µg ml⁻¹ polybrene (Aldrich). a, Control PC12 cells cultured in the above growth medium; b, PC12 cells incubated for 7 days in growth medium containing 50 ng ml⁻¹ of 2.5S NGF (Collaborative Research, Inc.); c, e, f, PC12 cells infected with Ki-, Ha- or Mo-MSV, respectively, and incubated for 4 days in growth medium; d, PC12 cells infected with the temperature-sensitive mutant of Ki-MSV and incubated for 4 days in growth medium at a non-permissive temperature, 39 °C. Scale bar, 100 µm.

usually died. Abelson murine leukaemia virus, carrying the *v-abl* gene, did not induce long processes, although the cells became spindle-shaped (not shown) and were easily detached from dishes after prolonged incubation. Simian sarcoma virus showed no apparent effect on the morphology of PC12 cells.

Under our culture conditions (see Fig. 1 legend), PC12 cells proliferated at an average doubling time of 42 h. By contrast, Ki-MSV-infected, process-extending cells stopped dividing within 3 days (Fig. 2). A similar result was obtained with Ha-MSV (not shown). The changes induced by the *v-ras*-containing MSVs do not seem to represent mere degeneration of the cells,

because the infected cells survive for many weeks, migrating actively. It was also observed that when PC12 cells were infected by Ki- or Ha-MSV and incubated for 24 h in growth medium, they could survive and extend processes in serum-free medium. The initial incubation in serum-containing medium seemed to be required for the expression of the phenotypic changes. However, cell division did not appear necessary (more rigorous experiments to explore this point are underway).

Several lines of evidence indicated that *v-ras* genes are responsible for these changes. First, the agent responsible for the changes was probably a sarcoma virus component in each virus stock, because helper MLV alone did not induce these changes, and, moreover, the number of process-extending cells induced by each of the stocks of Ki- or Ha-MSV from several different sources was roughly comparable to the number of focus-forming units measured on a rat fibroblast cell line (data not shown). It is known that only one protein is encoded by the genome of each MSV, that is, p21^{v-Ki-ras} by Ki-MSV and p21^{v-Ha-ras} by Ha-MSV¹¹. Second, transforming viruses carrying oncogenes other than *v-ras* did not induce the same type of changes (see above). Third, a temperature-sensitive (ts) mutant¹² of Ki-MSV failed to arrest the growth of PC12 cells at non-permissive temperature, although the process-inducing activity was not completely abolished (Fig. 1d). This last observation may suggest either that viral p21 proteins have more than one functional domain, or that growth-arrest requires more activity of viral p21 than does process-induction. Partial transforming activities of the ts Ki-MSV mutant at non-permissive temperature in other cell types have been reported^{4,13}.

To determine whether the changes induced by the *ras*-containing virus are involved in neuronal differentiation, we investigated whether neurone-specific properties known to be induced by NGF are also induced by the viruses in PC12 cells. First, we observed that Ki- or Ha-MSV-infected PC12 cells contained higher activities of acetylcholinesterase (as did NGF-treated cells¹⁴) than did uninfected control cells (Table 1). Second, electrophysiological examinations revealed that the mean resting potential of Ki-MSV-infected PC12 cells, as well as of NGF-treated cells, was significantly greater than that of untreated controls (Table 2). Moreover, Ki-MSV-infected, process-extending cells showed significantly higher levels of action potentials than did control cells. Judged from the two components of action potentials (that is, low and high threshold potentials), both Na⁺ and Ca²⁺ channels seemed to develop

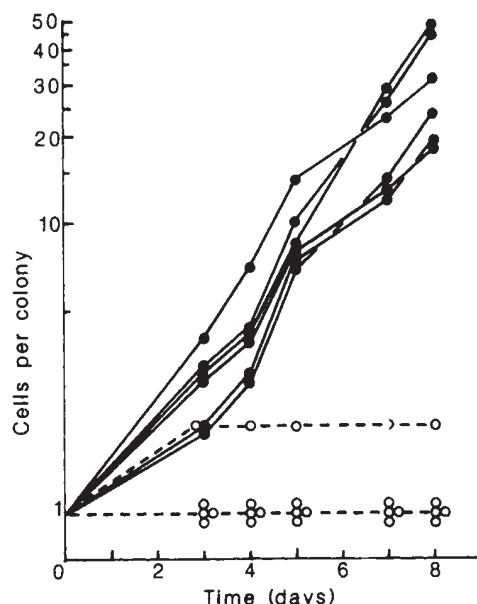


Fig. 2 Growth curve of Ki-MSV-infected or uninfected PC12 cells. PC12 cells were infected in suspension with Ki-MSV (MOI = ~1) and plated into a 96-well dish at ~1 cell per well with growth medium. As a control, uninfected PC12 cells were plated in the same way. After 16 h, wells containing a single cell were marked and the numbers of cells in each well were scored under a phase-contrast microscope at 24–48-h intervals. ●, Uninfected cells; ○, Ki-MSV-infected, process-extending cells.

after Ki-MSV infection, although to a lesser extent than those induced by NGF¹⁵ (Table 2).

The above results indicate that at least some of the properties associated with the induction of neuronal differentiation by NGF are also induced by Ki- or Ha-MSV in PC12 cells. This is in marked contrast to the previously known biological activities of these viruses *in vitro*, which include malignant transformation of certain cells^{1–5}, blockade of Ca²⁺-dependent differentiation of primary⁶ as well as established mouse keratinocytes⁷, and growth stimulation of differentiation-competent mouse erythrocytic cells⁸. We have observed that rat

Table 1 Acetylcholinesterase activities in PC12 cells treated with NGF or infected by Ki- or Ha-MSV

	Acetylcholinesterase activity (nmol per min per mg protein)		
Control	NGF	Ki-MSV	Ha-MSV
Expt 1			
39.6 ± 2.2	56.1 ± 3.3	55.4 ± 2.1	ND
Expt 2			
32.8 ± 0.7	42.7 ± 1.3	68.2 ± 1.1	46.5 ± 0.72
Expt 3			
a, In growth medium:			
24.5 ± 1.0	35.5 ± 0.8	28.1 ± 0.7	ND
b, In serum-free medium:			
ND*	44.2 ± 1.5	47.0 ± 1.6	ND

PC12 cells (3×10^5 cells per 60-mm dish) were infected with Ki-MSV (multiplicity of infection (MOI) ~1) and incubated for 5 days in growth medium (expts 1, 2, 3a) or 1 day in growth medium, then 4 days in serum-free Dulbecco's modified Eagle's medium (DMEM) (expt 3b). As controls, the same number of cells were cultured in growth medium with or without 50 ng ml⁻¹ of 2.5S NGF. The cells were collected and homogenized as described elsewhere¹⁴. A colorimetric enzyme assay was performed with homogenates from triplicate cultures for each group by the method of Ellman *et al.*²⁶. Data are given as mean ± s.e. ND, not determined.

* Control PC12 cells did not survive in serum-free medium.

Table 2 Electrophysiological properties of PC12 cells treated with NGF or infected with Ki-MSV

Control (n = 8)	NGF (n = 11)	Ki-MSV (n = 15)
Resting membrane potential (mV)		
-22.9 ± 1.0	-28.4 ± 0.33	-27.3 ± 1.6
Time derivative of action potential (V s ⁻¹)		
Low threshold (-20 mV):		
0	2.70 ± 0.84	1.21 ± 0.37
High threshold (0 mV):		
0.64 ± 0.43	3.89 ± 0.40	2.20 ± 0.45

PC12 cells (1×10^5) were infected in suspension with Ki-MSV and plated in 35-mm poly-D-lysine-coated plastic dishes with growth medium. The same number of uninfected cells were plated in medium with or without 50 ng ml⁻¹ NGF. After 8 days of incubation at 36 °C, the culture fluid was replaced with a recording buffer (130 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl₂, 0.8 mM MgSO₄, 5.5 mM glucose and 10 mM HEPES, pH 7.3), and electrophysiological examination using a microelectrode was performed as described elsewhere²⁷. Cells of typical sizes and morphologies were impaled and hyperpolarized to -100 mV for 1 s, then action potentials were triggered by injecting 50-ms depolarizing current pulses. Two components with threshold potentials of about -20 mV (representing activity of voltage-dependent Na⁺ channels) and about 0 mV (activity of voltage-dependent Ca²⁺ channel) were recognized in the time derivative of action potential, and are listed separately here. In control cells, a low-threshold action potential was not evolved. Results are given as mean ± s.e.

C6 glioma, a cell line whose origin was more closely related to that of PC12, was also growth stimulated by these MSVs (unpublished observation). Therefore, the growth-arresting and differentiation-inducing effects of Ki- and Ha-MSV may be highly specific to either this particular cell line or, possibly, to neuronal cells in general.

In experiments to investigate the mechanism of action of this phenomenon, we observed that anti-NGF serum of reasonably high titre failed to inhibit the process-extension and growth-arrest induced by Ki-MSV. Also, we have not detected any biologically active factors in conditioned medium collected from Ki- or Ha-MSV-infected PC12 cultures. We therefore believe, although this has not been proved, that some intracellular reactions induced by viral p21, rather than endogenously secreted differentiation factors, may trigger the phenotypic changes described above.

Very recently, two groups of investigators have discovered phenomena related to our observation. First, D. Bar-Sagi and J. Feramisco found that the transforming Ha-*ras* protein—but not its normal counterpart—introduced into PC12 cells via microinjection induces process-extension and arrest of DNA synthesis (personal communication), providing further evidence that the activated p21 itself is indeed responsible for this phenomenon. Second, Alemà *et al.* found that Rous sarcoma virus, which carries the *v-src* gene, induces changes almost identical to those described here in PC12 cells¹⁶. However, the kinetics of process-induction by *v-ras* and by *v-src* seem to differ from each other; *v-ras* induces processes with a shorter lag time and at a higher rate than either NGF or *v-src*. Although the significance of this difference and the relationship of these two analogous phenomena are unclear, one possibility is that *v-ras* and *v-src* share a common pathway of action in this system. Previously, we have found that two clones of flat revertants isolated from a Ki-MSV-transformed fibroblast cell line were resistant to 'retransformation' by *v-src*-containing viruses as well as by *v-ras*-containing viruses¹⁷, suggesting some functional relationship between the two oncogenes in transformation of fibroblasts also.

The mechanism of action of NGF remains controversial^{18,19}. Hypotheses have been proposed to take account of second messengers such as cyclic AMP and Ca²⁺ (ref. 20), as well as of other signal-transducing mechanisms²¹⁻²³ and direct translocation of the ligand-receptor complex into the cell nucleus^{18,19}. Examination of such early physiological changes in PC12 cells infected by either *v-ras*- or *v-src*-containing viruses should provide useful information on the mechanisms of action of the viral oncogenes as well as of NGF itself. To examine the role of cyclic AMP in this system is of special interest, in view of the recent discovery that the *ras*-related genes in yeast seem to serve as GTP-dependent regulatory components of adenylate cyclase^{24,25}.

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Deletion of Huntington's disease-linked G8 (D4S10) locus in Wolf-Hirschhorn syndrome

James F. Gusella*, Rudolph E. Tanzi*, Patricia I. Bader†, Mary C. Phelan‡, Roger Stevenson‡, Michael R. Hayden§, Karen J. Hofman||, Anne G. Faryniarz* & Kerin Gibbons*

* Neurogenetics Laboratory, Massachusetts General Hospital, Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA

† Parkview Memorial Hospital, Fort Wayne, Indiana 46805, USA

‡ Greenwood Genetics Center, Greenwood, South Carolina 29646, USA

§ Clinical Genetics Unit, University of British Columbia, Vancouver, British Columbia, Canada V6H 3V5

|| The Kennedy Institute, Johns Hopkins Hospital, Baltimore, Maryland 21205, USA

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterized by progressive involuntary movements and dementia^{1,2}. The symptoms of the disease, although devastating in severity, do not usually appear until the third to fourth decade of life. The gene defect is highly penetrant, and results in the loss of neurones in the basal ganglia, globus pallidus, and more diffusely in the cortex. A DNA marker, G8 (or D4S10), is tightly linked to Huntington's disease and this gene has been localized to chromosome 4 (ref. 3). The discovery of this linkage marker raises the possibility of developing a presymptomatic test for the disorder, and of eventually isolating the disease gene based on its map position⁴. We have now regionally localized the DNA marker G8 to the terminal band of the short arm of the chromosome, a region representing approximately 0.5% of the total human genome. The assignment was made by examining DNA from patients with Wolf-Hirschhorn syndrome, a birth defect resulting from partial heterozygous deletion of the short arm of chromosome 4.

Chromosome 4 contains approximately 6.55% of the total human genomic DNA. The search for additional DNA fragments near the HD gene and for the HD gene itself would be facilitated if the G8 marker were more precisely localized to only a small region of the chromosome. To achieve this goal, we used cells from patients afflicted with Wolf-Hirschhorn syndrome (WHS), a congenital anomaly involving heterozygous deletion of part of the short arm of chromosome 4 (4p-syndrome)⁵⁻⁷. Victims of WHS are profoundly mentally retarded and have a characteristic craniofacial appearance including microcephaly, arched eyebrows, ocular hypertelorism, broad malformed nose, lobeless ears and micrognathia. The loss of chromosome 4 material can be the result of a *de novo* deletion, or due to inheritance of an unbalanced translocation, but most commonly involves the terminal 4p16 band⁷.

Initially, we obtained blood samples from six independent victims of WHS to establish permanent lymphoblastoid cell lines, and to prepare genomic DNA. A fibroblast line, GM0343, from a seventh WHS patient was obtained from the NIGMS

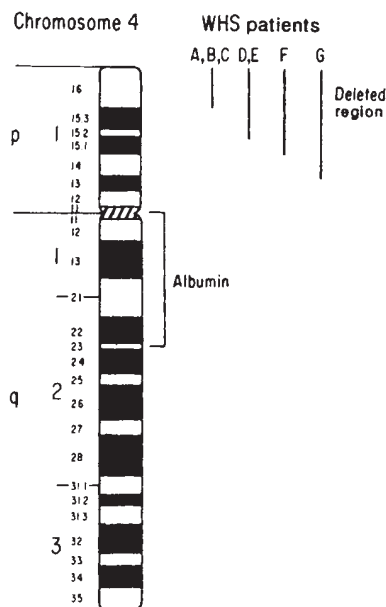


Fig. 1 Extent of chromosome 4 deletion in Wolf-Hirschhorn syndrome (WHS) patients. The region of chromosome 4 deleted in each patient, as determined by standard trypsin-Giemsa banding techniques, is shown. Note that the application of more sophisticated prophase banding methods could potentially detect differences between the deletions in patients A, B and C. The region containing the albumin locus is taken from ref. 17.

Human Genetic Mutant Cell Repository, Camden, New Jersey⁸. These patients presented a range of symptoms characteristic of WHS, and each had suffered an apparent terminal deletion of 4p. The extent of deletion in each WHS victim based on standard trypsin-Giesma banding is depicted in Fig. 1. In the case of patient D, the deleted 4p region had been replaced by an unidentified segment of another chromosome⁹. Although the size of the missing region was not identical in each case, all

patients were monosomic for at least band 4p16 which represents about 1/15 of the chromosome. For patients A, B and D, both parents were available for study. Blood samples could not be obtained from parents of the other cases. For patient A, an unaffected brother was also sampled.

The genetic linkage of G8 to HD was originally achieved using two restriction fragment length polymorphisms (RFLPs) detected by this probe in human genomic DNA digested with *HindIII*³. The informativeness of the G8 region as a genetic marker has recently been expanded by the cloning of adjacent genomic sequences and the discovery of additional RFLPs with the enzymes *BglI* (two sites), *EcoRI*, *PstI*, and *NciI* (ref. 10 and J.F.G., R.E.T. and R. A. Heft, manuscript in preparation). We reasoned that if the G8 locus mapped outside the deleted region, this could be demonstrated by detecting heterozygosity at any of these seven restriction enzyme sites. On the other hand, if the G8 region were deleted in a given individual, heterozygosity should never be observed. In addition, the apparent genotype of the WHS victim in such a case might be incompatible with the inheritance of one allele from each parent. We therefore performed Southern blot experiments with genomic DNA from each of the seven WHS patients, the three available sets of parents and the normal sibling of patient A. DNA was digested with each of the enzymes listed above, fractionated by agarose gel electrophoresis, transferred to a nylon filter and hybridized with appropriate probes known to reveal each RFLP. As a control, the DNAs were also typed for a *PstI* polymorphism at the albumin locus which maps below the centromere on chromosome 4¹¹. The results of this analysis are shown in Table 1.

Four of the seven WHS patients were heterozygous at the albumin locus indicating, as expected, that they were not deleted for this gene on the long arm of chromosome 4 and this polymorphism displays a level of heterozygosity of 49% in the general population. None of the seven patients was heterozygous at any of the RFLPs in the G8 region, although each of the normal parents was heterozygous for at least one of the seven sites. Table 1 also shows the level of heterozygosity displayed by each of these RFLPs in a random sample of 50 North Americans. Overall, 92% of unrelated individuals are heterozygous for at least one restriction enzyme site using this set of

Table 1 Phenotypes observed for RFLPs at the albumin and G8 loci

Individual	Cell line	Albumin locus <i>PstI</i>	G8 locus							Heterozygous at G8 locus
			<i>HindIII</i> (no. 1)	<i>HindIII</i> (no. 2)	<i>BglI</i> (no. 1)	<i>BglI</i> (no. 2)	<i>EcoRI</i> (no. 1)	<i>NciI</i>	<i>PstI</i>	
Patient A	GUS 2305	12	1	1	2	2	1	1	1	No
Father A	GUS 2302	12	1	1	1	2	2		12	Yes
Mother A	GUS 2303	12	1	1	12	2	1		1	Yes
Brother A	GUS 2304	12	1	1	1	2	12		12	Yes
Patient B	GUS 1548	12	1	1	1	2	1	1	1	No
Father B	GUS 1549	2	1	1	12	2	12		1	Yes
Mother B	GUS 1547	12	12	1	12	2	1		1	Yes
Patient C	GUS 1654	12	2	1	1	1	2	1	2	No
Patient D	GUS 2085	1	2	1	2	2	2	2	1	No
Father D	GUS 2225	12	12	12	2	2	2		1	Yes
Mother D	GUS 2224	1	12	12	2	2	12		1	Yes
Patient E	GUS 2098	1	2	1	1	2	2	2	1	No
Patient F	GUS 1553	12	1	1	2	2	2	1	1	No
Patient G	GM 0343	1	2	1	1	1	1	2	2	No
Heterozygosity		49%	46%	36%	46%	22%	58%	38%	16%	92%

Phenotypes for each RFLP were scored from the pattern of fragments observed on an autoradiogram after hybridization of Southern blots (containing genomic DNA digested with the indicated enzyme) to probes known to detect polymorphisms at either the albumin or the G8 locus (refs 3, 10 and J.F.G., R.E.T. and R. A. Heft, in preparation). Alleles are numbered 1 and 2 in order of decreasing size except in the case of *HindIII* site 2, where the order is reversed³. Heterozygotes at a particular site therefore have the phenotype 12. Heterozygosity in the general population, shown in the last line of the table, was calculated for albumin from ref. 16. For the G8 RFLPs, both individually and in combination, the table shows the level of heterozygosity observed in a random sample of 50 North Americans. All GUS cell lines are lymphoblastoid lines initiated in our laboratory for preparation of DNA³. GM 0343 is a fibroblast line purchased from the Human Genetic Mutant Cell Repository⁸.

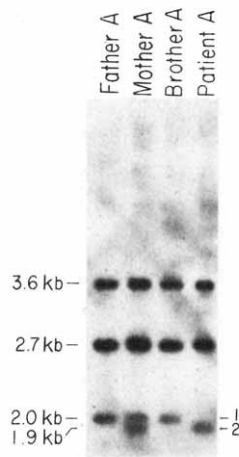


Fig. 2 Deletion of the G8 locus in patient A. DNA from patient A, his brother, and both parents was digested with *Bgl*I and fractionated by agarose gel electrophoresis (0.8% agarose, 3 V cm⁻¹, 18 h). The DNA was transferred to Zetapor nylon membrane (AMF/Cuno) and hybridized to ³²P-labelled probe R7¹⁰. R7 is a phage clone containing a 16-kb fragment of chromosome 4 that partially overlaps with the G8 phage insert. Together, the two clones comprise approximately 29 kb of adjacent sequence from chromosome 4 (J.F.G., R.E.T. and R. A. Heft, in preparation). Fragments representing alleles 1 (2.0 kb) and 2 (1.9 kb) are indicated on the figure. All other fragments detected by the probe are invariant.

seven RFLPs. The probability of picking seven individuals who are homozygous for all sites is therefore $(0.08)^7 = 2 \times 10^{-8}$. Thus, based on the lack of heterozygosity alone, it is likely that many or all of these WHS victims are in fact hemizygous at the G8 locus.

In patient A, the G8 locus has definitely been deleted from one chromosome (Fig. 2) as the child's DNA shows only the 1.9-kilobase (kb) *Bgl*I band representing the 2 allele. The genotypes of the father, mother and brother are 1 1, 1 2 and 1 1, respectively. Therefore, the WHS child is hemizygous at the G8 locus, having received a 2 allele from the mother with no contribution from the father. Hemizygosity at the G8 locus in patient A can also be demonstrated using the *Eco*RI RFLP (Table 1). For patients B and D where parental DNAs were also available, it was not possible to unequivocally demonstrate deletion of G8 by genotype alone. For these two, and for the patients with no available parents, we have compared the intensity of hybridization to sequences from the G8 region with that to a probe for albumin, which maps to the long arm of chromosome 4 (4q). Examples of these dosage experiments are shown in Fig. 3. A filter containing *Hind*III-digested DNA from patient B and his parents and patient F, was hybridized sequentially to pK082, a subclone of G8, and to pCHSA33-1, an albumin cDNA^{12,13}. *Hind*III was chosen for this experiment as both probes detect a fragment in the 3.7-kb range, thereby eliminating the possibility of artefact due to unequal transfer to the filter of DNA fragments of widely divergent sizes. In both patients, the intensity of hybridization of the albumin probe was approximately equal to that seen in the two normal parents. With G8, however, the intensity of hybridization was markedly reduced in the WHS children, indicating hemizygosity at the G8 locus. Similar experiments (data not shown) using *Hind*III and other restriction enzymes suggest hemizygosity at the G8 locus in each of the seven WHS victims. Ultimate confirmation of these results could be obtained by *in situ* hybridization experiments, or by segregating the deleted chromosomes in somatic cell hybrids. We are currently pursuing the latter strategy since it will generate cell lines with which new DNA probes can be quickly mapped to the vicinity of the HD gene.

The assignment of G8 to 4p16 does not dictate that the HD locus is also contained in this band. As the two are tightly linked,

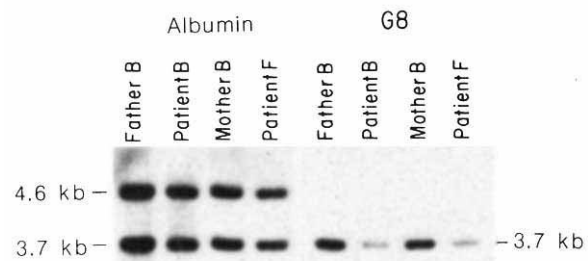


Fig. 3 Detection of hemizygosity for G8 locus by dosage. DNA (5 µg) from patient B, patient F, and the normal parents of patient B were digested with the enzyme *Hind*III and fractionated by electrophoresis on a 1% agarose gel. The DNA was transferred to Zetapor membrane and hybridized to ³²P-labelled pK082, a plasmid subclone of the G8 insert^{10,12}. This probe hybridizes to a single fragment of 3.7 kb in the size range examined. After autoradiography to detect the pK082 hybridization, the probe was removed from the filter by washing for 2 h at 65 °C in 0.01 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M Na-citrate). The filter was then hybridized to ³²P-labelled pCHSA33-1, a human albumin cDNA clone¹³. This probe detects two fragments of 4.6 kb and 3.7 kb. Both probes detect additional fragments >10 kb in a *Hind*III digest, but these were not compared in these experiments due to difficulties in obtaining uniform transfer of large restriction fragments. The fact that both probes detect small fragments of similar size (3.7 kb) allows direct comparison of relative dosage without the ambiguities introduced by comparison of fragments that are very different in size.

however, at a distance considerably less than 10 centimorgans (10% recombination), it is likely that the HD gene maps to either 4p16 or to the distal portion of 4p15^{3,4,14}. More precise assignment of the HD gene will probably result from the isolation of another polymorphic DNA marker on the opposite side of the disease gene from G8. Such a flanking marker would make it possible to bracket the HD gene and physical mapping of the new marker relative to G8 would then give a precise position for the HD locus⁴. The isolation of a flanking marker is also essential for the development of an accurate presymptomatic and prenatal linkage test for HD¹⁵. With only the G8 marker, it is impossible to eliminate potential errors in diagnosis that would be caused by the recombination events that can occur between the HD locus and G8. Choosing DNA fragments at random from chromosome 4 has thus far been unsuccessful in providing the needed flanking marker. The mapping of G8 to the region deleted in WHS victims has presented a new, more efficient strategy. Segregation of the deleted chromosomes from WHS patients in somatic cell hybrids will provide the material necessary to rapidly screen new DNA clones to determine whether they map to 4p16, which represents only about 1/15 of the chromosome. Selective hybridization schemes based on these cell lines might also yield large amounts of DNA from this band, and provide a tremendous boost for efforts to clone and characterize the HD gene itself. Given the proximity of the G8 and HD loci, at least some of the WHS patients, especially patient F, must carry a deletion and therefore be hemizygous for the normal counterpart of the defective Huntington's disease gene. If the HD mutation causes the inactivation of a gene, these patients might be the functional equivalents of an HD heterozygote with a clinical picture clouded by the effects of other hemizygous loci. Since WHS victims rarely live to an advanced age and HD is normally a late-onset disorder, it is not possible to make such a conclusion at this time. Long-term follow-up and careful neurological examination of older WHS patients who have been tested for deletion of the HD gene by DNA analysis might certainly be warranted, as would attempts to obtain neuropathological information on this syndrome. If the HD mutation does not cause inactivation of the gene, hemizygosity at this locus might still contribute in some way to the symptoms of WHS. A resolution of this issue will probably await

the further characterization of both disorders at the molecular level.

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Fission yeast *Schizosaccharomyces pombe* correctly excises a mammalian RNA transcript intervening sequence

Norbert F. Käufer*, Viesturs Simanis & Paul Nurse

Department of Cell Cycle Control, Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields, London WC2A 3PX, UK

Study of heterologous gene expression in the budding yeast *Saccharomyces cerevisiae* has shown that this organism is incapable of correctly removing intervening sequences from transcripts of higher eukaryotic genes¹⁻³. This is probably due to the stringent requirement for the presence of a TACTAAC box close to the 3' end of the intervening sequence if splicing in *S. cerevisiae* is to occur⁴⁻⁶. Comparison of the introns found in the fission yeast *Schizosaccharomyces pombe*⁷⁻¹⁰ has identified conserved sequences similar to those found in higher eukaryotes¹¹. Therefore, we have investigated whether *Schiz. pombe* is capable of accurately excising intervening sequences from the transcripts of higher eukaryotic genes. We show here that both the 5' and 3' splice sites of the simian virus 40 (SV40) small-T antigen transcript are accurately utilized when cloned viral DNA is expressed in *Schiz. pombe* cells. These data suggest that *Schiz. pombe* may be a better model system than *S. cerevisiae* for the genetic study of RNA splicing and for expressing higher eukaryotic genes.

The fission yeast *Schiz. pombe* is as convenient for molecular genetic analysis and gene manipulation¹² as the better-known budding yeast *S. cerevisiae*, but has been rather neglected in studies of eukaryotic gene expression. Recently it has become clear that *Schiz. pombe* genes generally have more introns than those of *S. cerevisiae* and that the conserved sequences in the vicinity of intron-exon boundaries are more similar to those found in higher eukaryotes. The consensus sequences found so far for *Schiz. pombe* introns are shown in Table 1. These are less stringent than those found in *S. cerevisiae*, particularly with respect to the TACTAAC box near to 3' splice sites, but are

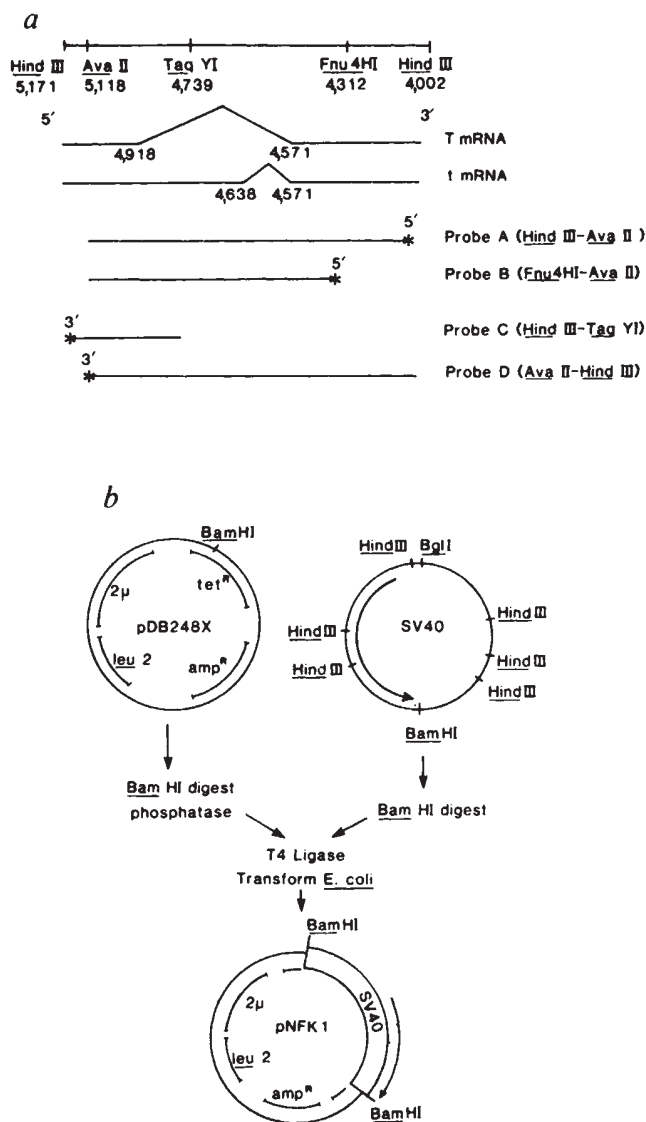


Fig. 1. a, Construction of plasmid pNFK1. The structure of the SV40 early region mRNAs within the 1,169-bp HindIII B fragment of SV40 is shown. The nature of the probes used in this study is also indicated. In each case, the site of labelling is given first. The numbering system used is that suggested in ref. 13. Asterisks indicate sites of labelling. b, Structure of SV40 early region mRNAs. pDB248X DNA was digested to completion with BamHI, treated with calf intestinal alkaline phosphatase to prevent self-ligation, and ligated to BamHI-digested SV40 DNA. The ligation mix was used to transform competent *Escherichia coli* DH-1 to ampicillin resistance. Tetracycline-sensitive clones were identified by replica plating and the DNA extracted from the transformants and analysed by digestion with *EcoRI* to determine the orientation of SV40 DNA in the plasmid. Standard techniques²³ were used throughout. All enzymes were purchased from Boehringer Mannheim and used according to the manufacturer's recommendation. The arrow on the SV40 genome indicates the direction of transcription of the early region. The structure of pNFK1 is shown. Similar results were obtained with pNFK2 in which the orientation of the SV40 DNA is reversed.

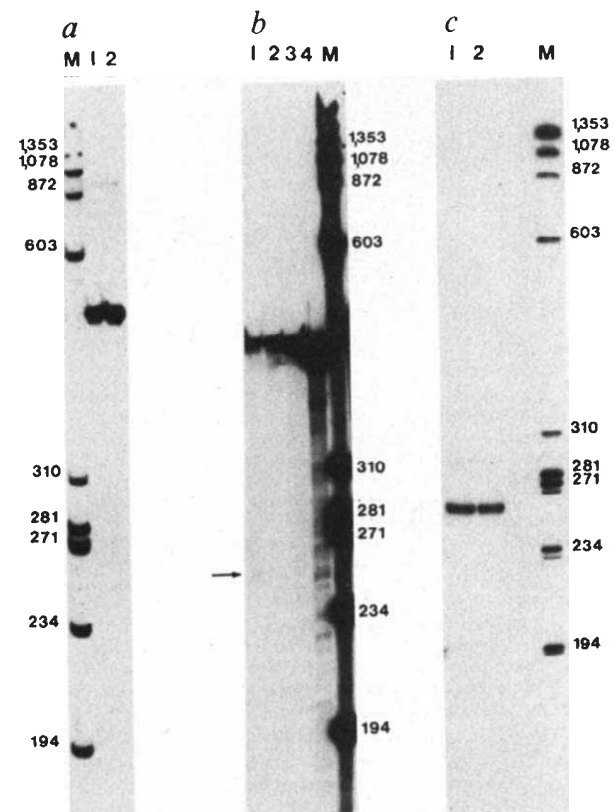
very similar to those observed in higher eukaryotes. Given these similarities, it is possible that transcripts from higher eukaryotic genes may be correctly spliced in *Schiz. pombe*.

To test this possibility, we have introduced SV40 DNA into *Schiz. pombe* and investigated the splicing of the early region, which encodes the small-T and large-T antigens. The messenger RNAs coding for these two proteins are produced by differential splicing of a precursor mRNA. They share the same 3' splice site but use different 5' splice sites (ref. 13 and Fig. 1a). SV40 DNA digested with BamHI was ligated into the *Schiz. pombe* shuttle vector pDB248X, and the resultant molecule, pNFK1 (Fig. 1b), was transformed into a *Schiz. pombe* leu1-32 h⁻ strain

* Permanent address: Institut für Biochemie und Molekular Biologie der Freien Universität Berlin, Ehrenbergstrasse 26-28, D-1000 Berlin 33, FRG.

Fig. 2 S_1 mapping of 5' and 3' splice sites of SV40 early region transcripts in *Schiz. pombe*. **a**, Mapping of 5' splice sites using probe D. Lanes 1, 2, 10 μ g poly(A)⁺ RNA from cells transformed with pNFK1, S_1 digestion at 37 °C and 22 °C, respectively. Exposure was for 16 h at -70 °C with preflashed film and intensifying screen. M, DNA size marker consisting of Φ X174, digested with *Hae*III and 5'-end labelled with T4 polynucleotide kinase. **b**, Mapping of 5' splice sites using probe C. Lanes 1-3, 20 μ g, 15 μ g and 10 μ g, respectively, of total RNA extracted from SV40-infected CV-1 cells; lane 4, 10 μ g of poly(A)⁺ RNA from cells transformed with pNFK1. S_1 digestion was at 37 °C. Exposure was at -70 °C for 14 days with an intensifying screen. The arrow indicates the position of the 257-nt band. **c**, Mapping of 3' splice site using probe B. Lanes 1, 2, 10 μ g of poly(A)⁺ RNA from cells transformed with pNFK1. S_1 digestion was at 37 °C. Exposure was overnight at -70 °C with an intensifying screen.

Methods. RNA was extracted from cells in mid-exponential growth as follows. Cells were collected by centrifugation, washed once in ice-cold buffer A (50 mM HEPES-NaOH, pH 7.9, 5 mM EDTA, 150 mM NaCl) and resuspended in buffer A at $\sim 2 \times 10^7$ cells ml⁻¹. An equal volume of acid-washed glass beads (0.5 mm diameter; Sigma) was added and the cells broken by vigorous vortexing for 2 min. The liquid was removed to a fresh tube and the beads washed three times with buffer A. The combined lysate and washings were centrifuged briefly to remove cell debris and the supernatant fraction, adjusted to 1% (w/v) SDS and 100 μ g ml⁻¹ proteinase K (Sigma). After incubation at 37 °C for 30 min, the sample was extracted twice with phenol and chloroform, and precipitated with ethanol. Polyadenylated RNA was selected as described elsewhere²³ by two rounds of affinity chromatography on oligo(dT)-cellulose. DNA was extracted from SV40-infected CV-1 cells 72 h after infection at a multiplicity of 10 plaque-forming units per cell²³. S_1 mapping was performed essentially as described elsewhere²⁴. Annealing was overnight at 48 °C in 65% formamide, using 50 ng of probe, typically 20,000-50,000 c.p.m. Digestion was for 30 min at the indicated temperature using 400 U ml⁻¹ S_1 nuclease (BRL). 5'-End-labelled probes were prepared using T4 polynucleotide kinase following treatment with alkaline phosphatase. 3'-End-labelled probes were prepared using avian myeloblastosis virus reverse transcriptase. Standard techniques for labelling and subsequent gel purification were used²³. Samples were analysed on 0.3-mm-thick polyacrylamide gels²⁵ which were fixed and dried before exposure.



by selecting for *leu*⁺ prototrophic transformants. Polyadenylated RNA was prepared from a culture of one of these transformants in mid-exponential growth. A Northern blot of this RNA was probed with the 1.1-kilobase (kb) *Hind*III B fragment of SV40 which covers the early gene region (Fig. 1a). Transcripts hybridizing to this probe were detected, demonstrating that the transformants contained SV40 DNA and that the early region was being expressed. Transcript mapping suggested that they were being initiated at the SV40 early promoter. Some premature termination of transcription was also observed. No transcripts hybridizing to this probe were observed using RNA from cells transformed with pDB248X (data not shown). To determine if the SV40-specific transcripts were being correctly spliced, S_1 nuclease mapping experiments were undertaken.

In order to map the 5' splice sites of the transcripts, a 1,120-base pair (bp) *Ava*II/*Hind*III fragment was 3'-end labelled at the *Ava*II site using reverse transcriptase (Fig. 1a, probe D). Two fragments should be protected from S_1 digestion if both 5' splice sites are used, one of 484 nucleotides (nt) corresponding to the small-T transcript, and one of 204 nt corresponding to the large-T transcript. The predominant band observed is at 484

nt (Fig. 2a), demonstrating accurate use of the small-T antigen mRNA 5' splice site. However, no band was seen at 204 nt, suggesting that the large-T donor site is used very inefficiently, if at all. Some very weak bands of higher relative molecular mass (M_r) were also seen on prolonged exposure, consistent with the presence of a small amount of unspliced RNA. To confirm this result a second, shorter probe was used. A 432-bp *Hind*III/*Taq*I fragment, 3'-end labelled at the *Hind*III site, was prepared (Fig. 1a, probe C). Full-length probe should be protected if the small-T antigen 5' splice site is used. To test whether the large-T antigen 5' splice site is used at all, the autoradiograph was overexposed to see if the predicted 257-nt band could be observed. Many weak bands became visible, one of which was 257 nt long; this band co-migrated with a protected fragment corresponding to the large-T antigen mRNA 5' splice site obtained using RNA extracted from SV40-infected CV-1 cells (Fig. 2b). This suggests that the large-T antigen mRNA 5' splice site may be used, although at an extremely low level.

To map the 3' splice site, which is common to transcripts of both large-T antigen and small-T antigen, a 810-bp *Ava*II/*Fnu*4HI fragment, 5'-end labelled at the *Hind*III site, was used as a probe (Fig. 1a, probe B). A 260-nt fragment was protected from S_1 -nuclease digestion (Fig. 2c), indicating that the 3' acceptor site is accurately used by *Schiz. pombe*. In addition to the 260-nt band, another, less intense band at 810 nt was observed corresponding to protection of full-length probe. This may be due to DNA-DNA reannealing or to a fraction of the transcripts not being processed. Correct use of the 3' splice site was confirmed using a *Hind*III/*Ava*II fragment 5'-end labelled at the *Hind*III site (Fig. 1a, probe A), when the expected protected fragment of 570 nt was observed (data not shown).

To determine whether the large-T or small-T antigen proteins were being synthesized, a Western blot of protein extracted from *Schiz. pombe* cells transformed with either pDB248X or pNFK1 was probed with pAb419 (ref. 14), a monoclonal antibody which recognizes an epitope found in the N-terminal region common to both proteins. Synthesis of the 20,000- M_r small-T antigen was clearly detectable in cells transformed with pNFK1 (Fig.

Table 1 Conserved sequences at intron-exon boundaries

	5' Splice site	Internal conserved sequence	3' Splice site
Higher eukaryotes	G GTAAGT G	CTAAT G C	TAG
<i>Schiz. pombe</i>	G GTANGT	CTAAT G C	TAG A
SV40 large-T	G GTATTT	CTAAT	TAG
SV40 small-T	G GTAAAT	CTAAT	TAG

The consensus for *Schiz. pombe* was derived from refs 7-10, 22 and that for higher eukaryotes from ref. 11. The SV40 sequence was taken from ref. 13.

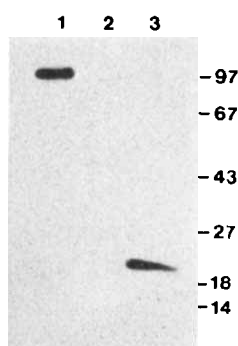


Fig. 3 Western analysis of proteins synthesized in *Schiz. pombe* transformants. Lane 1, 10 ng of purified SV40 large-T antigen; lane 2, protein extracted from cells transformed with pDB248X; lane 3, protein extracted from cells transformed with pNFK1. The numbers indicate the positions of marker proteins ($M_r \times 10^{-3}$).

Methods. *Schiz. pombe* cells transformed with the indicated plasmids were grown to a cell density of 5×10^6 cells ml^{-1} in minimal medium. The cells were collected by centrifugation, washed once in phosphate-buffered saline and broken by vortexing with glass beads. The broken cells were boiled in sample buffer²⁶ for 2 min. Cell debris was then removed by centrifugation, and the protein extracts were loaded onto a 5–15% (w/v) polyacrylamide gel²⁶. After electrophoresis the protein was transferred to nitrocellulose²⁷. The blot was hybridized with pAB419 (ref. 14) as described elsewhere²⁸. After washing, bound antibody was detected using ¹²⁵I-protein A (Amersham). The blot was autoradiographed using preflashed film and an intensifying screen at -70°C .

3). However, even on prolonged exposure, no large-T antigen was detected, a result consistent with the observed splicing pattern of the SV40 early region transcripts made in *Schiz. pombe*.

These results demonstrate that *Schiz. pombe* can correctly recognize the 5' and 3' splice sites of the SV40 small-T antigen pre-mRNA. The correct 3' splice site is not recognized in the budding yeast *S. cerevisiae* and the 5' splice sites utilized only very inefficiently¹⁵. In *Schiz. pombe* the 5' splice site used to produce the large-T antigen mRNA was used very inefficiently, if at all—an unexpected result, since in lytically infected monkey cells the large-T antigen 5' splice site is used two to three times more frequently than the small-T antigen 5' splice site¹⁶. This could be due to the difference in sequence between the SV40 large-T antigen 5' splice site and the *Schiz. pombe* consensus sequence (Table 1). None of the *Schiz. pombe* introns sequenced to date has a thymine at nucleotide 5 at the 5' end. Alternatively, it is conceivable that although *Schiz. pombe* can recognize higher eukaryotic splicing signals, it cannot cope with multiple 5' splice sites for a single 3' splice site. Thus, having identified the 3' splice site, perhaps through an interaction with the internal conserved sequence, the *Schiz. pombe* cell may scan through the transcript until it identifies the first 5' splice site and completes intron excision. A second 5' splice site beyond this would then be used inefficiently, if at all. In the SV40 system, this possibility is readily testable using the $\Delta 54$ –59 mutants of SV40¹⁷, in some of which the small-T antigen 5' splice site is deleted.

Another observation which may be relevant to this phenomenon is the report that *Xenopus* oocytes use only the small-T 5' splice site when SV40 genomes are co-injected with antibodies against ribonucleoprotein particles¹⁸. This observation suggests that the two 5' splice sites may have different properties. It is thus possible that use of the large-T 5' splice site may have a different requirement for ribonucleoprotein particles from that of the small-T 5' splice site which cannot be provided by the heterologous *Schiz. pombe* cells.

This is the first report of a higher eukaryotic intron being correctly spliced in yeast. Although it may initially seem odd that fission yeast should behave so differently from budding yeast, it should be remembered that these two organisms, although both ascomycetes, are not closely related. Recent sequence comparisons suggest an evolutionary divergence which could be as much as 1,200 Myr¹⁹. There are also differences in their promoters²⁰ and in aspects of the mitotic cycle such as the

presence of a G_2 period and chromosome condensation²¹. In all cases, the behaviour of *Schiz. pombe* is closer to that of higher eukaryotes than is *S. cerevisiae*. For these reasons we suggest that *Schiz. pombe* should be considered more seriously as an organism for expressing higher eukaryotic genes and for genetic analysis of eukaryotic splicing.

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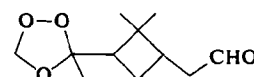
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Corrigendum

Hydroxymethyl hydroperoxide and bis(hydroxymethyl) peroxide from gas-phase ozonolysis of naturally occurring alkenes

S. Gäb, E. Hellpointer, W. V. Turner & F. Korté *Nature* **316**, 535–536 (1985)

THE structure of an ozonide used to clarify the reaction pathway was drawn without a necessary methyl group. The product in equation (10) should have the following structure:



Erratum

Similarity of vaccinia 28K, v-erb-B and EGF receptors

H. R. Chen & W. C. Barker *Nature* **316**, 219–220 (1985)

IN this item of Scientific Correspondence on similarities of receptor proteins, ref. 5 should be shown on line 6 of the second paragraph. In addition the authors' address was omitted. It should read: Protein Identification Resource, National Biomedical Research Foundation, Georgetown University Medical Center, 3900 Reservoir Road, N.W. Washington, DC 20007, USA.

Sedimentary microrhythms

WITH regard to the interesting article by House¹ in which sedimentary microrhythms from marine Jurassic strata of southern England were investigated, the following may be of additional interest.

House assigned 60 ammonite zones to the Jurassic period, of which two were referred to the non-marine Lulworth Beds (Purbeck Beds). In the Purbeck Beds of Dorset and the Weald 39 cycles have been identified among the ostracod faunas which Anderson and Bazley² have termed faunicycles. One faunicycle comprises an alternation of two ostracod assemblages: (1) species identified as tolerant of relatively saline waters (S-phase); (2) species tolerant of brackish to almost freshwater conditions (C-phase). The ostracod genus *Cypridea* dominates the C-phase of the faunicycles. The number of ammonite zones (among other biostratigraphical tools) allocated to the Lulworth Beds (without ammonites) will ultimately depend on the positioning of the internationally agreed base of the Cretaceous period. In southern England the Cinder Bed Member is generally accepted as the base of the Cretaceous although recent work questions some assumptions³⁻⁵. For the calculations here the Cinder Bed faunicycle² is taken as representing the base of the Cretaceous.

If the Lulworth Beds are accepted as representing two ammonite zones, Table 1 shows values for the average length of a faunicycle (based on 20 faunicycles). The assignment of two ammonite zones to the Lulworth Beds may prove to be inaccurate and lead to re-interpretation of the values shown (for example, one ammonite zone for 20 faunicycles would give averages approaching the obliquity figure; M. R. House, personal communication). Anderson and Bazley² considered that if the faunicycles were climatically induced, the precession of the equinoxes might have been responsible.

Sedimentary microrhythms can be recognized in the Lulworth Beds, and evidence of possible annual seasonal rhythmites can be seen in the deposits of restricted pools (Lower Dirt Bed of central Dorset) and also expressed in the pattern of fossil tree-growth rings⁶. There is much sedimentary variation in the Purbeck Beds due to their deposition marginal to a lagoon of fluctuating water level⁷.

The faunal cyclicity of the Purbeck Beds reflects palaeoecological cyclicity that is

not mirrored by lithological microrhythms. Is it coincidence that this cyclicity averages 117 kyr, assuming the Jurassic to be 70 Myr in duration? Is this an expression of the 100-kyr cycle due to orbital forcing⁸? Ostracod assemblages have allowed correlation across north-west Europe in the late Jurassic and early Cretaceous⁹, consisting of groups of ostracod zones and faunicycles. The faunicycles are assemblages of ostracod species; not purely evolutionary sequences, they represent changes in palaeoenvironment and may be laterally variable in their specific content². The fact that the faunicycles persist over rapid lateral and vertical lithological/environmental changes suggests the involvement of some longer-term factors in the cyclicity. Might it be the case that, in palaeoenvironmental settings where sedimentary microrhythms are not manifestly obvious, such as in the marginal-marine Lulworth Beds of Dorset, biotic rhythmicity (ostracod faunicycles) reflects climatic cyclicity due to orbital forcing?

Note added in proof: Anderson¹⁰ has recognized one more faunicycle in the Purbeck Beds. Following assumptions made above the average faunicycle length is 111 kyr.

MICHAEL R. SANDY

Department of Geology
and Mineralogy,
Marischal College,
Aberdeen University,
Aberdeen AB9 1AS, UK

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Pre-Rhaetic therian mammals

THE discovery of a *Kuehneotherium* tooth in deposits older than the Rhaetic transgression in Somerset, UK, was reported by Fraser, Walkden and Stewart¹, who claim that it is the oldest record of a therian mammal, predating the kuehneotheriids from the supposedly 'Rhaetic' locality of Saint-Nicolas-de-Port in eastern France, described by Sigogneau-Russell². However, one of us has recently studied the amphibian and reptile remains collected at Saint-Nicolas-de-Port by G. Wouters and P. Coupatez, and has compared this faunal assemblage with those from better known German localities³. This reveals that the Saint-Nicolas-de-Port fauna is of roughly the same age as that from Kuhn's levels 15-20 of the Halberstadt locality⁴, and should thus be referred to the Norian.

Therefore, the Emborough *Kuehneotherium* and the mammals from Saint-Nicolas-de-Port may actually be of the same age, and may all qualify as the oldest known mammals.

The case of the Saint-Nicolas-de-Port locality illustrates the fact that the Rhaetic stage is of very doubtful value as far as continental faunas are concerned. The Rhaetic was first defined as the top of the marine Triassic in the northern Alps, but even there its validity has been questioned, as its fauna is not significantly different from that of the Norian, so that many stratigraphers have abandoned it altogether⁵. Outside the Alpine domain, the term Rhaetic is even less meaningful, especially in the Germanic domain where correlations are difficult. It seems to be impossible to define a typically Rhaetic vertebrate fauna, and vertebrate localities lumped together in the so-called Rhaetic may be of very different ages. We are therefore in full agreement with the opinion expressed by Fraser, Walkden and Stewart¹ that the Emborough find "throws doubt on the fundamental distinctiveness of pre-Rhaetic and post-Rhaetic vertebrate faunas". Continued use of the terms Rhaetic and Rhaetic in a stratigraphical sense causes more problems than it solves, and cannot contribute to a better understanding of the relative stratigraphical position of late Triassic continental vertebrate localities.

ERIC BUFFETAUT
MICHEL MARTIN

Laboratoire de Paléontologie
des Vertébrés,
CNRS UA 720,
Université Paris VI,
4 place Jussieu,
75230 Paris Cedex 05,
France

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FRASER, Walkden and Stewart¹ have recently announced the discovery of fossil mammal teeth in a terrestrial fissure deposit at Emborough in the Mendip Hills (Somerset, UK). It has been dated by them as 'pre-Rhaetic' following Robinson² who, in the then absence of direct palaeontological dating, invoked comparisons based on rock types, topographical position and the general nature of the faunal assemblage. We disagree with this.

Robinson's use of rock types for dating is inappropriate, since fissure fills and normal stratified sequence are of markedly different origin. The marl lithologies cited by Fraser *et al.*¹ as typical "pre-Rhaetic" are also known from the "Upper Rhaetic"³.

We envisage a situation where, in the prolonged period of sub-aerial exposure before, or during, the early Rhaetic, tectonic and solution features formed which

Table 1 Average length of a faunicycle

Duration of Jurassic (Myr)	Duration of each zone (Myr)	Average faunicycle length (kyr)
60	1.00	100
65	1.08	108
70	1.17	117
75	1.25	125

contained little sediment. As sea levels rose during the Rhaetian, these fissures filled with contemporaneous sediment, with additional solution occurring during intermittent periods of local Rhaetian sub-aerial exposure⁴.

When Robinson² dated the Emborough fissure as Norian she was influenced by the absence of mammals, so that their discovery removes this age connotation. Fraser *et al.*¹ distinguish a discrete Norian fauna, in spite of the lack of an independent Norian date for this supposed pre-Rhaetic reptile assemblage, and in disregard of the evidence from the Tytherington fissure⁵ which is dated as Rhaetian using diverse microfloras. Fraser *et al.*¹ dismiss this date, indicating incorrectly that these particular deposits were tectonically disturbed and mixed. Microfloras of Rhaetian age have now been recovered⁶ from six separate fissure fills in Tytherington Quarry and except for one minor slump sequence none of these palynomorph-bearing intervals shows any evidence of tectonic mixing. Whilst some of the Tytherington fissures were tectonically initiated⁶, most show a pre-infill solution influence or are entirely solutional in origin. In addition to microfloras, five of these fissures also contain fish (including *Pholidophorus*, *Gyrolepis*, *Hybodus*, *Saurichthys*) and crustacea (*Euestheria minuta*) characteristic of the Rhaetian⁶. The microflora and fauna both occur in the same matrix as the supposed pre-Rhaetic Norian reptile assemblage.

Because the Tytherington 'Norian' reptile fauna has been shown to be Rhaetian and because the nature of Robinson's evidence for a pre-Rhaetic age at Emborough is highly equivocal, we suggest that the presence of *Kuehneotherium* could more reasonably indicate the date of the Emborough deposit than vice versa. It is generally accepted that the abundant mammal fossils (including *Kuehneotherium*) from other British fissure fills are of Rhaetio-Liassic age⁷. We therefore place the Emborough deposit in the Upper Rhaetian and see no justification for the date proposed by Fraser *et al.*¹.

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D. I. WHITESIDE

29 Colegrave Road, Bloxham,
Oxon OX15 4NT, UK

J. E. A. MARSHALL

Department of Geology,
The University, Highfield,
Southampton SO9 5NH, UK

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FRASER, WALKDEN AND STEWART
REPLY—We are most interested to learn that on the basis of detailed comparisons

with well-dated German faunas¹, Buffetaut and Martin would now revise downwards the age of the Saint-Nicholas-de-Port vertebrate assemblage. Although there is as yet no other firm evidence to confirm a Norian rather than Rhaetian age for these late Triassic deposits, the presence of *Kuehneotheriids* amongst otherwise acceptable Norian forms does suggest a mammalian occurrence as old as the Emborough *Kuehneotherium* specimens.

As stressed by Warrington *et al.*², the term 'Rhaetic' in Britain is a lithostratigraphic one, not to be confused with the chronostratigraphic stage name 'Rhaetian' which generally extends a little lower. The marine incursion which established characteristic Rhaetic deposits in Britain was a rapid event affecting wide areas almost simultaneously³. It thus provides a useful stratigraphical marker, and it would be difficult to persuade geologists not to use it. Hitherto, all records of late Triassic mammals have post-dated this transgression, but at Emborough, where we have found mammal remains for the first time, the isolated Triassic pocket containing them lies beneath a planation surface associated with Rhaetic (Westbury Formation) sediments. The pocket sediments themselves are completely distinct, and have continental characteristics identical with Norian deposits nearby. We fully agree that in many other places, including parts of mainland Europe, such a distinction could not so clearly be made.

Meanwhile, Whiteside and Marshall claim, on the basis of their own interpretation, that the Emborough deposit in which we have found mammalian teeth is probably of Rhaetian rather than Norian age. Our dating of the deposit followed careful re-assessment in the field as part of a wider study of the fissure deposit phenomenon, and our acceptance of Robinson was arrived at because we found no cause to criticize Robinson's evidence. Certainly, if palynological evidence came to light, the deposit could prove to be Rhaetic, but equally it might be pre-Norian or mid-Jurassic.

The evidence for a pre-Rhaetic age is based on classical stratigraphical argument, reviewed by Whiteside and Marshall, and no evidence to the contrary has emerged. The deposit is in fact closely similar to local Keuper Marl facies and is comparable to pre-Rhaetic boulder and conglomerate deposits elsewhere in the Mendips. It contains no evidence of derived Rhaetic or post-Rhaetic sediments (including the ubiquitous phosphatic basal bone-bed formerly exposed nearby within Emborough Quarry) and is unlike any of the known post-Norian lithologies in the area. If Whiteside and Marshall believe that the deposit belongs to the Upper Rhaetian, then the onus of proof lies with them and we will welcome any new information they can provide.

As regards the dating of the Tythering-

ton deposits, we merely wish to sound a strong note of caution at a locality where the fissures owe their origin to tensional stresses which continued into the Rhaetic. Whether directly attributable to tectonic dilation, or resulting from meteoric re-working or marine flooding of open systems (for example, ref. 4) the effects of remobilization, mixing and contamination can be very subtle. We would not dispute that the fish faunas may be of Rhaetian age, but it would be helpful if the full sedimentological and faunal details of Tytherington were published in order to provide a firmer basis for discussion. However, the age of the Tytherington assemblages has no direct bearing on the age of the Emborough mammal find, and we raised the matter originally because if it does prove to be Rhaetic it further supports our contention (misunderstood by Whiteside and Marshall and by the author of ref. 5) that there is no longer any case for a fundamental distinction between the terrestrial faunas before and after the Rhaetic transgression based on the presence or absence of mammals.

N. C. FRASER*
G. M. WALKDEN
V. STEWART

Department of Geology
and Mineralogy,
Marischal College,
University of Aberdeen,
Aberdeen AB9 1AS, UK

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Flow law for ice in polar ice sheets

DOAKE and Wolff¹ have argued that a relationship between strain rate $\dot{\epsilon}$ and stress τ of the form $\dot{\epsilon} = A\tau^n$ with $n = 1$, rather than the commonly used value of 3 (and where A is the flow law constant), provides a better fit to data from polar ice masses. Weertman² has discussed the implications. I remain unconvinced for the following reasons.

(1) The Devon Ice Cap borehole is within a distance of 3 ice thicknesses from the ice divide. Modelling of flow near divides, with $n = 3$, shows that the shape of the velocity-depth profile changes with distance; tilting data, if analysed by Doake and Wolff's method, would give a value of n increasing from zero near the divide to 3 at about 10 ice thicknesses from it³. Doake and Wolff's analysis is therefore invalid.

(2) At Camp Century, the strain rate measured for $\tau_{xz} = 10$ kPa, where x_z^1 and 3 define a rectangular coordinate system, is less than the observational error⁴. The same is almost certainly true at Law Dome.

* Present address: Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK.

Doake and Wolff's diagrams suggest that removal of this point would increase n to ~ 2 in both cases.

(3) The Amery Ice Shelf data lie on the line for $n=3$, not $n=1$.

(4) The measured spreading rate of Ward Hunt Ice Shelf ($\dot{\epsilon}=3.3 \times 10^{-12} \text{ s}^{-1}$, $\tau=12 \text{ kPa}$)^{5,6}, not mentioned by Doake and Wolff, lies between the lines for $n=1$ and 3. A significant fraction of this ice shelf consists of old sea ice, so its impurity content will be high. Because soluble impurities 'soften' ice, correction for this would move the data point towards the line for $n=3$. It has been suggested that the ice shelf may be grounded beneath the survey site, in which case the analysis would not apply. I believe that grounding can be ruled out because the surface slope is of the magnitude ($1.6 \times 10^{-3} \text{ rad}$) expected on floating ice⁵, and the slope is towards the Ward Hunt ice rise⁵; it would be in the opposite direction if the site were on the ice rise.

(5) Holdsworth⁷ suggested why the low-stress points from Erebus Ice Tongue deviate from the line $n=3$; crevasses penetrate a significant fraction of the thickness in the outer part and effectively soften the ice. This seems plausible to me.

(6) New data⁸ from Ross Ice Shelf give $n=3.3$.

(7) Surface velocity measurements^{9,10} on ice rises give values of n of 3.13, 3.7 and 4.2 over the stress range 50–130 kPa.

Doake and Wolff believe that borehole tilting is affected by transient creep because flow changes the depth of, and thus the shear stress on, an ice particle. Weertman suggested that this might make the flow law appear linear. This effect should be greatest in the ablation area of a temperate (0°C) glacier; the vertical velocity there is an order of magnitude greater than in polar ice sheets. Moreover, accumulation and ablation change the ice thickness seasonally by about 5 m. However, tilt measurements¹¹ in 12 boreholes in temperate glaciers provide convincing evidence that n lies between 3 and 4.

Measured velocity-depth curves vary widely in shape, even after allowance is made for temperature variations. I believe that differences in longitudinal stresses, which affect the shear strain rate only if the flow law is nonlinear, can account for much of the variation.

W. S. B. PATERSON

Paterson Geophysics Inc.,
Box 303, Heriot Bay,
British Columbia,
Canada VOP 1H9

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DOAKE AND WOLFF REPLY—Our position regarding the flow law for polar ice¹ (relating strain rate $\dot{\epsilon}$ and stress τ by $\dot{\epsilon}=A\tau^n$) is consistent with Lliboutry and Duval², who comment that "any attempt to determine the creep law of polycrystalline ice is illusory". Because factors such as crystal size and fabric, and impurity content appear to have a major role in determining the deformation of polycrystalline ice, and because these factors cannot, at least at present, be deduced or modelled *a priori*, there seems to be little point in using a complex flow law that requires large but unknown *ad hoc* corrections to be made to fit experimental data. Our approach has been to find the simplest relationship between observed strain rates and deduced stress field that produces the greatest degree of harmony and the lowest range of 'enhancement factors' between different data sets. It is also reassuring to note that Lliboutry and Duval² have independently concluded that, for the Holocene ice at Byrd Station, a linear flow law gives the best fit to field-tilting data.

As to the points raised by Paterson:

(1) Raymond's³ model of flow near ice divides shows that for $n=1$, there is no difference in the velocity profile shape with distance from the divide. An $n=1$ exponent gives a good fit to Paterson's observed velocity-depth profile at Devon Island, while $n=3$ with Raymond's model applied to Devon Island gives a poor fit⁴.

(2) There is common agreement that borehole data are difficult to analyse because of measurement errors and uncertainties. Discarding the point at 10 kPa at Camp Century would in any case lead to a value of $n=1.47$. There is no justification for discarding the point at Law Dome. The measured strain rate (before our adjustment to -28°C) is well above any likely observational error. (The data at Law Dome must in any case be constrained by the requirement of non-negative basal velocity, which led to the two circles (not used in the regression) at the high stress end of our Fig. 2d. Lliboutry and Duval² have produced more detailed arguments about factors affecting flow in boreholes, but we reiterate that if $n=3$ is used, such large variations in A (the flow law constant) are required to fit data that the flow law ceases to have any predictive value.

(3), (4) The analysis by Thomas⁵ of ice shelf data assumed that resistive forces due to shear stress gradients could be ignored. This is not true for either Amery Ice Shelf (where also the site was "situated on a small hill"⁵) or Ward Hunt Ice Shelf (where also grounding may "extend beneath the site"⁵). In reply to the letter by Hattersley-Smith⁶, we do not read Weertman's comments as saying that our data go against a linear creep law.

(5) The only data available from an ice shelf which approaches the ideal

'unbounded' state are those from Erebus Glacier Tongue⁷. It seems unnecessary to postulate a 'softening' of the ice by crevasse penetration, which in any case still does not bring the data to the line $n=3$.

(6) The back-stress pattern⁸ on the Ross Ice Shelf was inferred from an otherwise untested theory of crevasse formation on the underside of ice shelves. Data are sparse and Jezek⁸ has reported discrepancies of up to 1 bar with values of back stress calculated by other methods⁹. Measurements of strain rate and crevasse height were made at different sites, meaning that interpolations had to be made¹⁰. No allowance for temperature variations have been made^{8,10} although this could "represent an approximately threefold variation in the strain rate" (ref. 9) for $n=3$. There is a large scatter in the data of Jezek and others¹⁰, even for the points selected as representing 'best data'. Although this is an intriguing use of unusual experimental data, we do not regard the values as being sufficiently accurate for defining a flow law.

(7) We specifically excluded discussion of ice rise measurements because of the approximate nature of their analysis.

The penultimate paragraph broaches the point that n appears to lie between 3 and 4 for temperate ice. We specifically confined our remarks to polar ice and did not consider temperate ice because of the problem of accounting for free water in the flow law². Because of this water, we see no reason why temperate and polar ice should have similar flow laws.

The final paragraph mentions velocity-depth curves in boreholes. We have shown that if measured temperature profiles are taken into account, better agreement is reached with $n=1$ than $n=3$ in polar ice. There are still discrepancies, which may be due to unmodelled factors such as impurity content or crystal size and orientation; these discrepancies are much reduced for $n=1$. We explicitly allowed for longitudinal stresses in the analyses represented by our equation (1) (ref. 1), but there are no data on their variation with depth. Again, we conclude that on evidence available it is not necessary to use a nonlinear flow law to describe the flow and deformation of polar ice sheets.

C. S. M. DOAKE
E. W. WOLFF

British Antarctic Survey,
Natural Environment Research
Council, Madingley Road,
Cambridge CB3 0ET, UK

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Mad White Giant: A Journey to the Heart of the Amazon Jungle. By B. ALLEN. Macmillan, London: 1985. Pp.249. ISBN 0-333-39316-3. £9.95.

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Nuclear Arms Control: Background and Issues. COMMITTEE ON INTERNATIONAL SECURITY AND ARMS CONTROL. NATIONAL ACADEMY OF SCIENCES. National Academy Press: 1985. Pp.378. ISBN 0-309-03491-4. £19.95.

Nuclear Energy: A Sensible Alternative. By KARL O. OTT and BERNARD I. SPINRAD (eds). Plenum: 1985. Pp.386. ISBN 0-306-41441-4. Np.

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Philosophical Papers, 1 and 2. 1: Human Agency and Language. 2: Philosophy and the Human Sciences. By CHARLES TAYLOR. Cambridge University Press: 1985. 1: Pp.294. ISBN 0-521-26752-8, hbk £25, pbk £7.95. 2: Pp.340. ISBN 0-521-26753-6, hbk £25, pbk £8.50.

Cell populations re-examined

from R. F. Jarvis and D. Rossington

Cell biology has been in existence for over 400 years. But only recently has it been possible to study cells in massive numbers.

"... these pores, or cells, were not very deep, but consisted of a great many little boxes

I no sooner discern'd these, which were indeed the first microscopical pores I ever saw, and perhaps that were ever seen but methought I had with the discovery of them, presently hinted to me the . . . reason of all the Phenomena of cork; . . ."

(Robert Hooke, 1665)

AND so an era began. The basic living unit of plant, animal and man, had been seen and identified. With the Dutch draper Leeuwenhoek's observation, at about the same time, of a central body within salmon blood cells, such observations heralded the beginnings of modern cell biology.

Within the confines of a tiny space (the average mammalian cell is some 15–20 μm in diameter) is a completely automated, computerized factory possessing an organizational capacity to direct the reproduction of cells into masses of tissues with unique tasks, so that a complete body can be built from a single isolated cell.

The living cell may appear fairly simple on the surface but microscopists have long recognized internal structures. The most prominent of these structures, the nucleus, houses the chemical templates of production, the chromosomes. The nucleus is suspended in cytoplasm, a rich fluid containing many complex molecules and many small organelles, and the whole is contained within an outer cell wall, the membrane.

Soon after fertilization the ovum begins to divide continuously to form a mass of

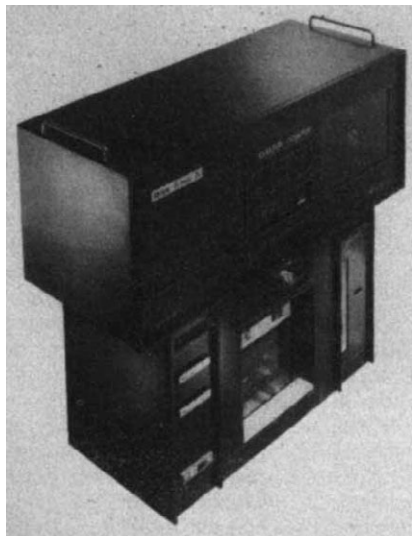


Fig. 1 An automated blood cell counter.

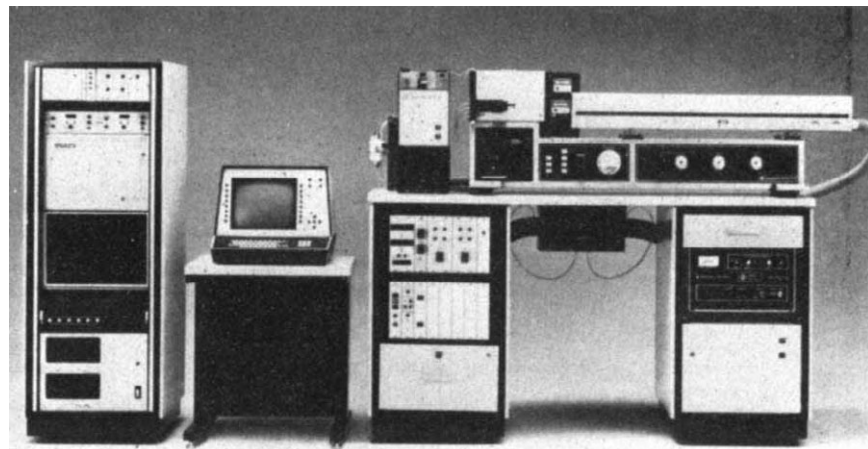


Fig. 2 A typical flow cytometer, using scattered light to determine cell characteristics.

smaller, similar cells. Within this mass of cells, or blastocyst, new cells are produced, and soon the three basic tissues of the fetus — the ectoderm, mesoderm and endoderm — emerge. The layers become entwined and growth continues, with the ectoderm further specializing to produce the skin and nervous system while the liver, pancreas, lungs and other organs, plus the digestive tract, spring from the primitive endoderm. Muscle, blood, bone and the connective tissues will result as the mesoderm divides and specializes.

Depending on the function of the tissue from which a cell is extracted, cells will vary in outer size, shape and internal organization. By studying the morphological and biochemical profiles of the cells, biologists can ascertain the family tissue and, often, whether the cell is healthy.

Methods of examining and classifying cells go back many years. The chemical and physical structures of the cell are routinely viewed after suitable processing to stain the various constituents with a range of organic dyes. Solid tissues are sliced by a sharp knife, or microtome, after the tissue has been held firmly embedded in a wax block or frozen solid. Then, the one-cell-thick section is coated onto a glass slide and stained. Many stains exist to show the presence of the internal structures of the cell and of particular cellular enzymes and both internal and external excretions.

The examination of blood to identify and count the red cells, white cells and platelets has found increasing importance as electrical methods have increased the precision and usefulness of such tests.

Most patients seeing doctors and entering our hospitals routinely have a full examination of their blood to aid in the diagnosis and treatment of their ailments. Apparatus exists to count and size, rapidly and routinely, the main types of blood cells. Blood specimens can be measured at rates of over 100 per hour, and data presented include the haemoglobin content as well as the concentration of several subpopulations of white cells. One such apparatus is shown in Fig. 1.

A new ability to study subpopulations of blood cells and suspensions of dispersed solid tissues has recently been developed, allowing cell surface molecules to be identified. The surfaces of circulating blood cells are not smooth but have many molecules projecting from them. The white blood cells are a vital part of the human immune system that defends us against the challenge of microorganisms which threaten to destroy us in order to live themselves. Although the main types of white blood cells can be routinely distinguished by morphology using manual staining, or automatically counted on a blood cell counter, our understanding of them and hence of the immune system has leapt forward in recent years because of the development of reagents which can identify parts of the cell surface molecules. With these monoclonal antibody reagents it has been possible to divide the white cell population responsible for specific immunity (the lymphocytes) into various subgroups based on the different molecules (or antigens) present on their surface. The complex task of correlating these subgroups according to function and

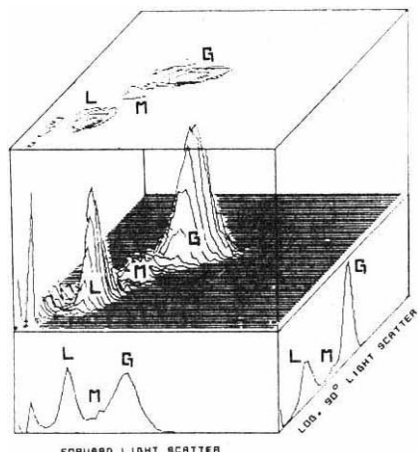


Fig. 3 The distribution of lymphocytes, monocytes and granulocytes displayed from simultaneous measurements of forward angle light scatter and wide angle light scatter as recorded by a flow cytometer, measuring 2,000 cells per second.

role is far from complete but this technique has already led to a better understanding of the various diseases of the immune system.

The circulating blood cells are also studied by haematologists who use commercial monoclonal antibody reagents in the same cell surface techniques to type and count the various immature or mature cells that are present in leukaemia and lymphoma. Here again, the ability to identify subpopulations has led to better diagnosis and monitoring of these tumours of the blood. Modern instruments, called flow cytometers, are now available that will examine many thousands of individual cells each second, and report on their characteristics. A typical flow cytometer is shown in Fig. 2. The cells in suspension are made to follow a confined trajectory through a very intense beam of light. Light is scattered from a cell entering the beam and transmits vital information to sensitive detectors concerning the size and constitution of the cell (as for example, in Fig. 3). Additionally, probes which fluoresce in the bright light can be used to identify particular antigens and other cellular constituents.

New methods in the field of cellular biology will emerge in the future. Pattern recognition computers are already able to evaluate a stained section much as a human does but, at present, are relatively slow. New identification probes will be devised to look inside the cell and evaluate its integrity. Understanding the functions of specialized cells, in relation to those surrounding them, will become increasingly important. The search for knowledge about these basic units of life will go on so that our understanding and treatment of abnormal tissues will continually improve. □

R.F. Jarvis and D. Rossington are at Coulter Electronics Ltd, Northwell Drive, Luton, Bedfordshire LU3 3RH, UK.

Cells in the laboratory

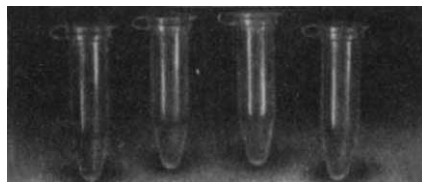
A selection of equipment—some to be seen at the Atlanta meeting of the American Society for Cell Biology.

- Using the direct immunofluorescence staining method, Dako's conjugated rabbit antibody against human IgG (F202) demonstrates the presence of immunoglobulin deposits in the basement membrane zone at the dermal epidermal junction. Positive staining for IgG in certain skin biopsy sites can be a useful tool for distinguishing systemic (SLC) from discoid (DLE) lupus patients. Other helpful applications for this conjugate include the indication of skin disorders such as dermatitis herpetiformis and bullous pemphigoid.

Reader Service No. 100.

- Bioglass reusable microcarrier beads, fabricated from a plastic latex base with a thin layer of glass on the outside are ideal for cell attachment and for easy harvesting. They can be made in a range of specific gravities and sizes for cell culture and viral vaccine applications. Their near neutral buoyancy means easy suspension and slow stirring speeds. Bioglass microcarriers can be cleaned and sterilized like ordinary glass and reused up to 10 times with no degradation of cell growth capacity. European distributors and stockists for Bioglass microcarriers are European Business Associates of Luxembourg.

Reader Service No. 101.



Colourful tubes from Elkay.

- Elkay's expanded range of 1.5 ml microcentrifuge tubes now offers a choice of two materials and three colours. The range includes 19 different types of microcentrifuge tubes ranging in size from 250 µl to 1.9 ml. Precision moulded from polypropylene or polyethylene, an integral plug cap insures that the tube can be conveniently sealed to eliminate the risk of spillage. This feature also makes the microcentrifuge tube safer for transportation and storage as well as centrifugation. The tubes are available in blue, green and yellow for easy sample identification. These 1.5 ml microcentrifuge tubes can withstand the high centrifugal force generated by modern centrifuges.

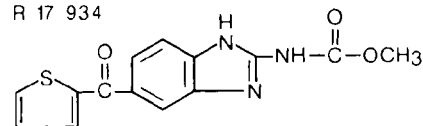
Reader Service No. 102.

- A new synthetic microtubule inhibitor, R46846, has been synthesized by Janssen Research Laboratories. The *cis* isomer of tubazole-C, this reagent inhibits the rate and extent of rat brain tubulin polymeriza-

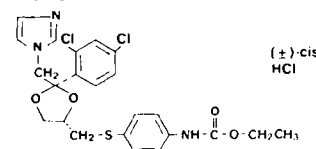
These notes are based on information provided by the manufacturers. To obtain further details about these products use the reader service card bound inside the journal.

tion with an ID_{50} of 3×10^{-7} M—about three times as potent as nocodazole and 12 times as potent as colchicine. The novel antimicrotubule compound R17934 (see below) is also new from Janssen, and as it

R 17 934



(R 46 846)



(±)-*cis*
HCl

is chemically unrelated to the familiar microtubule-disintegrating alkaloids (colchicine, vinca and so on), it should provide a useful alternative in studies of microtubule involvement and function.

Reader Service No. 103.

- The Falcon Labware Division of Becton Dickinson has introduced newly designed tissue culture dishes called Easy Grip. With the Easy Grip feature, the lid and dish can be picked up and stacked easily and cleanly regardless of hand size. The design also keeps the contents undisturbed when removing the lid. Many researchers have claimed the design of conventional dishes makes the handling of the dish a difficult procedure and the contents are occasionally disturbed during the process. The first Falcon tissue culture dish to receive this design is the 35mm size.

Reader Service No. 104.



Getting to grips with the Falcon Easy Grip.

- Oncor Inc. has now developed reagents and protocols for *in situ* hybridization. Any 32 S- or 3 H-labelled probe may be used in this system for the detection of DNA or RNA target in cells or frozen tissue deposited on microscope slides. DNA or RNA targets are detected *in situ* under the microscope after autoradiography. Counter stains such as haematoxylin and eosin and Wrights stain are compatible with the system. All Oncor probes are available labelled with 3 H or 32 S for use with the *in situ* hybridization kit.

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The Accurate Chemical & Scientific Corporation range includes growth factors and ECM coating.

• Now available from Accurate Chemical of Westbury, New York, extracellular matrix (ECM) coating was developed expressly by IBT of Israel for simulating *in vivo* cell growth in an *in vitro* system. As a naturally produced substrate, ECM plays an active role in the control of cell proliferation and differentiation, and should provide greater study potential in cancer and haemostasis research, biopsy specimen growth, and other areas of clinical application. The culture ware is supplied pre-coated with ECM. Also new from Accurate is fibroblast growth factor, a stimulant of growth in tissue culture.
 Reader Service No. 106.



Cytometry mechanized on the ACAS-470.

• The ACAS 470 workstation from Meridian Instruments functions as a "no-flow cytometer" that brings methods of automated clonal selection and fluorescence analysis to cell monolayers maintained in a tissue culture environment. This is accomplished through the integration of laser technology, epi-illumination microscopy and rapid, high resolution stage positioning — all under computer control. The intensity of the focused laser beam can be rapidly modulated to provide variable illumination for fluorescence excitation, photobleaching, cell surgery and cell killing. Fluorescent emissions are detected by a photomultiplier and digitized for storage. Results are presented on a colour graphic terminal. Coordinate locations of individual cells can be stored for automated time-based multiple analyses.
 Reader Service No. 107.

• Beckman Instruments, Inc.'s Model L7-55 ultracentrifuge combines simple operation, reliable induction drive and built-in diagnostics with an attractive price. The induction-drive ultracentrifuge can run all Beckman high-performance fixed angle, vertical tube and swinging bucket rotors. Model L7 offers speed to 55,000 r.p.m. and forces to 408,000g for everyday applications. It operates at 2° to 20°C with thermistor temperature sensing.
 Reader Service No. 108.

• Becton Dickinson has developed a new fluorescence activated cell sorter, the FACStar. This instrument incorporates the FACS cell sorter concept into a new user friendly design. The FACStar analyses cells or other biological particles, at the rate of ten thousand per second, using a laser beam. These cells are characterized on the basis of forward-angle light scatter, 90° light scatter and two-colour fluorescence. Individual cells of interest may then be physically sorted or separated. Operation of the FACStar is computer controlled. Single-key commands allow complicated analysis and sorting procedures to be performed without further operator intervention. The FACStar can also be operated manually so that performance can be quickly optimized without recourse to time-consuming software menus.
 Reader Service No. 109.

• The new electron microscope from Philips, the CM 10, is aimed at life scientists requiring advanced instrument control techniques. Central to the CM 10 technology is the Microcontroller, a microprocessor-based microscope control system which links the operator console and the column hardware. It enables control elements to be assigned general functions rather than directly connected to specific lenses or deflection coils. Consequently, each function, such as image focusing, always occurs with the same function knob, regardless of microscope mode or the magnification applied. Operator communication with the Microcontroller is via an interactive screen. Microscope mode and observation conditions are labelled in the screen as are the functions of special CM 10 softkeys, through which both mode and conditions are determined.
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• A new service from Molecular Biosystems provides custom-modified DNA. Modified oligonucleotides are available at 10–40 base units in length with a non-isotopic label. The modified SNAP oligonucleotides contain thymidine analogues with linker arms attached at C-5; such linker arms terminate in a primary amine or label of choice such as fluorescein, rhodamines or biotin. Amine-derivatized oligomers can be attached to solid supports for immobilization of hybrids. Labelled oligomers can be used for non-isotopic detection of complementary sequences.

Reader Service No. 111.

• Auto-Count, from Artek Systems Corporation, is a new low-cost automatic counter for rapidly counting bacterial colonies. The AutoCount has an adjustable aperture which allows operators to isolate an object or group of objects for separate image counting. Simple adjustment of its size discriminator dial elimin-



Bacterial counting by Artek.

ates objects that are smaller than the determined size threshold. The AutoCount can also be manually adjusted to a proper sensitivity threshold. Every scanned object is flagged with an illuminated dot so the operator can see exactly which objects have been included in the count. Objects can be virtually any shape, clearly defined or hazy, dark or light. Artek is a subsidiary of Dynatech Corporation and the Auto-Count is available from Dynatech in the United Kingdom.

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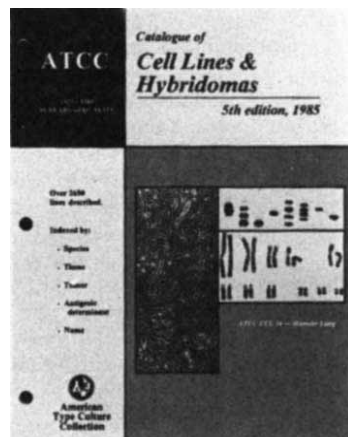
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Reader Service No.48



ATCC's catalogue now available from ATCC, 12301 Parklawn Drive, Rockville, MD.

• The fifth edition of the ATCC Catalogue of Cell Lines and Hybridomas has just been published, describing over 2,650 lines. The 305-page catalogue provides data on human, animal and plant cell lines; details on more than 200 hybridomas; a listing of cell lines involved in patenting; recommended media and growth conditions for many cell lines; and formulae for standard cell culture media and reagents. Lines are indexed by species, tissue cell type, tumour type, monoclonal antibody specificity, special applications (including virus studies) and originator's designation. Copies of the catalogue are available free of charge in the United States. Shipments outside the US will be charged \$6.50. ATCC is exhibiting its range at the meeting of the American Society for Cell Biology, Atlanta.

Reader Service No. 113.

• The Coulter D10 Th is a semiautomatic diluter designed to provide dilution of platelet rich plasma (PRP), for use with the Coulter Thrombocounter. The D10 Th can be operated in any one of the three modes which all control the same dilution function. By pressing the black touchplate on the front of the instrument, by utilizing the optional pipettor, or by the convenient foot switch. All three operating modes enable the D10 Th to aspirate, and then precisely dilute 3.3 µl PRP sample in 10 ml of Isoton 11, all in eight seconds.

Reader Service No. 114.

• Ricin A and B chains are now available from ICN Biochemicals. Whole ricin is a highly toxic protein of molecular weight 66,000 and is isolated from castor beans. The A chain is the enzymatically active subunit which interferes with protein synthesis, and the B chain binds to carbohydrate moieties and mediates entry into the cell. The A chain is virtually devoid of any toxic effects for whole cells as long as it is not attached to the B chain of ricin through sulphide bonding. The ricin A chain has been bound to monoclonal antibodies, hormones and numerous other cell surface ligands. Applications of conjugated ricin A include histological cell identification and removal of unwanted normal cells from suspensions.

Reader Service No. 115.

Non-radioactive DNA label

THE Orogenics DNA Chemiprobe kit was incorrectly described as a radioactive labelling system in *Nature* of 12 September. In fact the innovation is that Chemiprobe is a non-radioactive system for DNA labelling. For more details on Chemiprobe write in number 99 on the Reader Service Card.

• Bioscreen is a new automatic and compact analyser system from Labsystems. Using vertical photometry, the system measures microorganism growth. Practical applications are possible in many fields: food and beverages, cosmetics and toiletries, pharmaceuticals, water analysis, industrial effluents monitoring and basic biological and toxicological research. Bioscreen is fully automatic with a capacity for 200 simultaneous individual tests. Each testing cuvette is only 400 µl, therefore decreasing reagent consumption. And, the Bioscreen liquid dispensing system is easily autoclavable. Bioscreen consists of an analyser unit, a desk-top computer with customized software programs, a printer and disposables. The analysing unit itself contains a dispenser/diluter and an incubator connected to a photometer. The system sets on most any laboratory counter. During the test process, the computer reads information such as the initial cell population size, cell growth, growth speed and cell generations.

Reader Service No. 116.



Polaron's latest microcomputer-controlled tissue processor.

• Polaron Equipment's new E9200 automatic tissue processor employs a bench-top microcomputer for system control, instead of the electromechanical card reader previously used. The use of the computer provides much more flexibility and is much easier to operate than the older system. The reagent delivery system incorporates eight separate reagent storage bottles, each with its own peristaltic pump. The E9200 handles all reagents from initial osmication to 100% resin embedding and is capable of processing up to 126 samples at one time.

Reader Service No. 117.

• A new product data sheet from Schleicher & Schuell gives specifications for its Uniflo sterile, disposable laboratory syringe filters. Made with low protein-binding, pure cellulose acetate membranes, the 25-mm Uniflo filter offers high sample recovery, no extractables, and a process volume of up to 150 ml for materials as viscous as media with 10% bovine serum albumin. For each of the four available pore sizes (0.2, 0.45, 0.8, and 1.2 μm), a specifications chart gives flow rate, pressure rating, average hold-up volume, and application areas.

Reader Service No. 118.

• Nalge Company, a division of Sybron Corporation, has available a free wall chart to simplify the selection of the right filter for the job. This new four-colour chart includes complete selection and applications data for reusable filter holders and presterilized, disposable filter units, syringe filters and membranes. The chart also includes selection information for Nalgene 25-mm syringe filters, 47-mm membranes, and reusable holders.

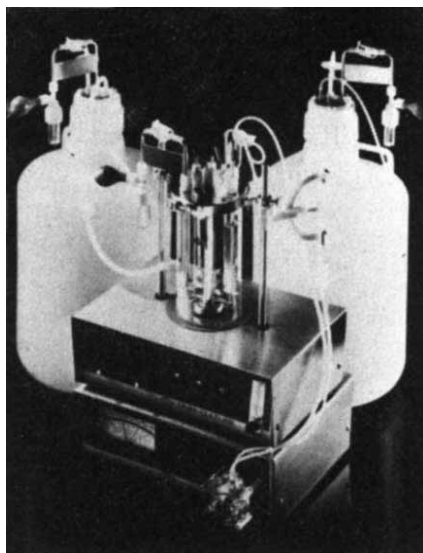
Reader Service No. 119.

• Bellco Biotechnology's new modular magnetic stirring system, the Sci/ERA quad drive, provides precision control of cell growth parameters for up to four remote stirring units from a single master controller. The one-to-nine position stirrer modules accept spinner flasks up to eight litres in size permitting convenient system expansion. Remote units are connected to the master controller via 10-foot heavy duty cables with locking plugs, allowing ample room to pass into or through incubators. System options include a sealed gel-electrolyte battery to protect cell cultures against power loss.

Reader Service No. 120.

• The PSS-80 automatic peptide synthesis system from Applied Protein Technologies Inc. features a unique in-line reaction monitoring system, fifteen randomly accessible, refrigerated amino acid reservoirs and a negative pressure delivery system. APT's Custom Peptide Synthesis Service furnishes peptides promptly in quantities of 10 mg to 10 grams or more. All are sequenced to demonstrate their purity and integrity. APT's proprietary chemicals include PEP-TIES which are insoluble solid-supports for immobilizing peptides and proteins. For polypeptide isolation and purification, PEP-SEPS are amino acid specific immobilized reagents. PEP-SEPS facilitate the design of DNA probes by isolating specific peptides or peptides containing specific amino acids. APT products can be seen on Booth 326 at the Atlanta Cell Biology meeting.

Reader Service No. 121.



Available from Techmation, the new VirTis chemostat.

• The VirTis Omni-Culture Chemostat bench-top fermentation system for continuous culture experimentation and aerobic/anaerobic studies is now available from Techmation. The system is designed to provide accurate, reproducible data, facilitating scale-up; it also regulates temperature, agitation, filtered air or gas flow and the transfer of medium. To maintain sterility throughout prolonged research studies, all components coming into contact with fermentation liquids are made of materials that are non-toxic, non-corrosive and repeatedly autoclavable.

Reader Service No. 122.

• A new vibration isolated bench from Photon Control of Cambridge is designed for electrophysiologists, neurophysiologists, cell biologists and molecular biologists using precision micromanipulators and probes of micrometre or sub-micrometre geometry. Users of the Model BT Series of biotechnology benches claim an ability to routinely investigate small cells (5 μm radii) with longer impalement times, and a substantial improvement in

signal to noise ratios. The BTT bench top is constructed from synthetic granite which consists of 96% solid granite aggregates bonded together, the remaining 4% is a special well damped matrix material.

Reader Service No. 123.

• The Skatron Microwash I and II 96-Well Automatic Microplate Washers have been designed for both enzyme immunoassay and radioimmunoassay applications; the versatile Microwash II has additional facilities which make it suitable for use with cell monolayers. The microplates are washed by selecting one of the three wash programs which permit either 3, 4 or 5 repetitions of the wash/aspirate cycle. The rinse program is used to prevent blockages caused by the build-up of crystalline deposits by flushing the wash liquid from the instrument at the end of the working day.

Reader Service No. 124.

• A new data sheet on the Kontes Micro Ultrasonic Cell Disrupter is now available. Designed to be capable of disrupting samples in a reproducible manner, the disrupter's LED output indicator provides a precise measure of the load imposed by a sample; previously documented responses can be confirmed to be re-occurring. Tip displacement amplitude is maintained by Kontes' monitor circuitry which assures stability by continuously correcting amplitude variations.

Reader Service No. 125.

• Optomax Inc. has introduced two new image analysis systems. System V is an automatic system utilizing dual microprocessors and high-speed measuring circuitry. Provision of circular and rectangular frames, variable in size and position, plus user-drawn frames, allows areas of interest within the image to be defined. In addition to a single detector, System V features a twin 'grey slice' detector and also a programmable 256 grey level auto detector. Vids V is a high resolution semi-automated system utilizing a graphics tablet and IBM PC or compatible computer. Input from the TV camera is viewed on the display monitor and features to be measured are 'drawn round' or 'dotted' using a hand-held cursor.

Reader Service No. 126.



A compact Optomax image analyser.

A seller's market for graduates

from Richard Pearson

Against the trend in other sectors, the demand for new graduates in scientific and technical subjects in Britain is strong.

EARLY last year the *Employment* column forecast that a revival in the job prospects for new qualifying graduates was imminent. In the event the upturn in recruitment has been faster and more widespread than anticipated, with employers from sectors as diverse as oil and chemicals, manufacturing, retailing and financial services all reporting growing numbers of vacancies. While the high fliers and information technology specialists have been in particular demand, graduates in the pure sciences and the applied sciences have also been beneficiaries. In 1984 the number gaining employment in industry and commerce was already 25 per cent higher than during the labour market doldrums of 1980-81 and is set to increase again in 1985. The one no-growth area is for jobs in higher education itself.

The International Labour Office (ILO) has recently highlighted the booming UK graduate labour market, and reports that it is not mirrored in most other countries. In the United States over half of new PhDs cannot find the type of employment for which they were trained. Unemployment among chemists and chemical engineers, for instance, was at its highest level for a decade in 1983. In West Germany, the number of jobless university graduates has quadrupled during the past four years to a total of over 115,000. Teachers, engineers, natural scientists, lawyers and economists were particularly hit. In France there were nearly 60,000 members of managerial and supervisory staff without jobs in early 1984, while in Switzerland, joblessness among young university degree holders has risen from 2 to 5 per cent in recent years — much faster than the general unemployment rate. Japan offered a mixed picture. Job offers for 1984 university graduates increased by 4.7 per cent as compared to 1983 thanks to a new phase of industrial innovation combined with an economic upturn; however,

openings for high school graduates were expected to decline by 12.9 per cent.

A common thread everywhere is that women graduates face more difficulties than men in finding and keeping a professional job, partly because a larger proportion of women take degrees in less "marketable" subjects, but also due to sex discrimination. In addition, the ILO report concludes, more and more professional workers are being forced to step down on the employment ladder and accept jobs for which they are overqualified, setting off a chain reaction which affects the entire labour market.

The final figures for the United Kingdom in 1984 show that of the 30,000 graduating in science subjects from the universities, nearly two-thirds went into employment, 15 per cent went onto further research and about 6 per cent went into some form of vocational training, the latter percentage having continued to fall sharply in recent years. Of particular note are the declining numbers entering teacher training; since 1980 the number has fallen by nearly one-third, and totalled just over 1,100 in 1984, of whom less than half were mathematicians or physicists. This means that the already serious shortages of mathematics and physics teachers is likely to get worse over the next decade. About one in eleven scientists were still seeking work six months after graduation. Of those scientists entering permanent employment, over half went into manufacturing industry, and about 14 per cent, a steadily declining fraction, entered the public sector. Less than four per cent got jobs in the education section while twice as many went into accountancy. In terms of actual jobs, the most common destinations were management services, and research and development and associated support activities. The pure scientists were more likely to go into further research or training, and also had higher

unemployment rates than the applied scientists, while more of the latter went directly into employment (Fig.1). Women were more likely to enter teaching than their male counterparts. The data for the polytechnics to be published shortly are expected to show broadly similar patterns.

On the salaries front the average for those starting jobs in autumn 1984 was about £7,000 per annum, with many companies in the growth sectors of oil and chemicals, electronics and information technology offering nearer £8,000 per annum. Salaries for 1985 are expected to be about £500 higher, with a few of the top payers in central London offering starting salaries in excess of £9,000 per annum as they seek to attract the "best" talent. Post-graduates with relevant degrees or experience relevant to the job being filled can command substantially higher salaries; most, however, receive salaries little higher than first degree graduates.

In the scramble to attract the "best" people, where, in the case of non-technical jobs employers often judge personal criteria before academic criteria, the traditional gentlemanly calm of the "milk round" is becoming outdated. In past years, virtually all the companies visiting colleges did so in the spring term, apart from the accountancy companies who were able to plead special circumstances and start their recruitment in the autumn term. Nowadays more companies start their recruitment campaigns before Christmas to try and catch the best students, and some are offering previously unheard of inducements such as "signing-on fees" if jobs are accepted quickly.

At the same time many students are becoming more confident of their job prospects and are concentrating on their examinations during their final year. They are delaying job search until after they have qualified, sometimes then taking a break of several months. In this way the graduate recruitment round, formerly concentrated into the spring term, is starting earlier, and extending later. If the anticipated growth in demand for graduates is matched by the expected fall in the numbers graduating then graduates who are well qualified, in both personal and academic terms, will be able to dictate not only the type of job they enter but also the time of year they seek work. □

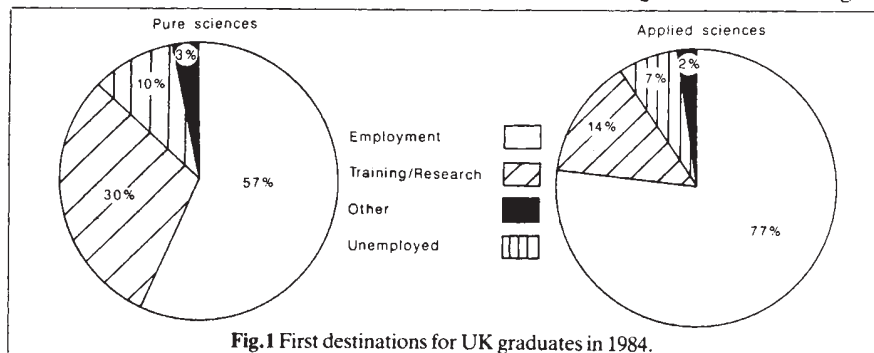


Fig.1 First destinations for UK graduates in 1984.

Richard Pearson is at the Institute of Manpower Studies, Mantell Building, University of Sussex, Brighton BN1 9RF, UK.